Ormia depleta: LABORATORY MAINTENANCE, STRAIN IDENTIFICATION, AND EVALUATION OF *Aphis nerii* AS A BANKER SPECIES

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ORMIA DEPLETA:
LABORATORY MAINTENANCE, STRAIN IDENTIFICATION,
AND EVALUATION OF *APHIS NERII* AS A BANKER SPECIES

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Ormia depleta (Wiedemann) is a tachinid fly used in the biological control of *Scapteriscus* spp. mole crickets. Several changes to the current method of rearing *O. depleta* in the laboratory were attempted. Manipulations of temperature, humidity, and photoperiod during the pupal and adult stages showed that the percentage of gravid females was higher at 23°C than at 27°C and at 70% relative humidity than at 85%, but photoperiod did not have a significant effect. A diet of melezitose did not increase the fecundity of flies compared to a melezitose-free diet. Increasing the number of planidia used to inoculate host mole crickets increased the number of pupae produced, but reduced the survivability of those planidia and the mean weights of the pupae produced. Larger host mole crickets were also found to produce larger pupae. *Acheta domesticus* was tried as a factitious host and found to be unsuitable. Although pupae were produced, they were small and few in number. Dissections of hosts with developing larvae showed that the

larvae in *Acheta* developed slowly and apparently had trouble emerging from the host. Identification of two Brazilian strains of *O. depleta* was performed by gas chromatographic analysis of cuticular hydrocarbons. The results indicated that, using the model used in this experiment, the strains could be identified with 80% confidence. An evaluation of the honeydew of *Aphis nerii* on its milkweed host indicated that it is an adequate carbohydrate source for *O. depleta*, but further study is needed in this area before any recommendations can be made.

CHAPTER 1
REVIEW OF LITERATURE

Four species of mole crickets are currently established in Florida. Three of these are immigrant pests of the genus *Scapteriscus*: *S. vicinus* Scudder (the tawny mole cricket), *S. abbreviatus* Scudder (the short-winged mole cricket), and *S. borellii* Giglio-Tos (the southern mole cricket). These three species can be differentiated by several features including dactyl spacing and the characteristics of the male song (Nickle and Castner 1984). All three *Scapteriscus* spp. mole crickets are believed to have arrived from South America around the year 1900 (Walker and Nickle 1981). *S. vicinus* and *S. borellii* are thought to have initially arrived in the United States through Brunswick, Georgia, in 1899 and 1904, respectively (Walker and Nickle 1981, Nickle and Castner 1984). *Scapteriscus abbreviatus* apparently arrived several times between 1899 and 1923 into several ports in Florida and southernmost coastal Georgia (Walker and Nickle 1981). Since then, *Scapteriscus* mole crickets have become major agricultural pests throughout Florida and into Georgia, Mississippi, South Carolina, and other states (Frank et al. 1998). They are the most damaging pests of turf and pasture grasses in Florida and are also pests of vegetable and field crops (Reinert and Short 1980). Bahiagrass, *Paspalum notatum* Fluegge, is apparently the preferred, and therefore most damaged, plant species (Reinert and Short 1980). Damage is typically caused by either the tunneling of the mole crickets, which exposes the roots to desiccation, or by direct feeding on the roots (Reinert and Short 1980). Traditionally, these pests have been controlled through the use of chemical pesticides such as carbaryl, chlorpyrifos, acephate, bifenthrin, fipronil,

imidacloprid, and others (Buss et al. 2002). Pesticide use is only a short-term solution because most of Florida's over three million hectares of bahiagrass are in low-maintenance areas such as pasture and roadside (Short and Reinert 1982), providing an unlimited supply of mole crickets during flight periods in September-October and April-May (Walker et al. 1992a). However, because multiple treatments are required to achieve adequate levels of control (Parkman et al. 1996) and because of other concerns raised by pesticide use, alternative methods of control have been sought.

At least four biocontrol agents have been considered for control of immigrant mole crickets, three of which have already been released in the field. The entomopathogenic nematode *Steinernema scapterisci* Nguyen and Smart has been effective against mole cricket populations, with infected individuals being found up to eight years in pastures after initial treatment and twelve years on golf courses after initial treatment (Frank et al. 1999, Frank et al. 2002). *Steinernema scapterisci* shows a much higher specificity toward its mole cricket hosts than do other *Steinernema* species (Nguyen and Smart 1990) and has had a measurable effect on mole cricket populations in treated areas (Parkman et al. 1994). The sphecid wasp *Larra bicolor* F. has been released and established in Florida, and its population is spreading in northern Florida. However, little research has been done on its effectiveness to date. The bombardier beetle *Pheropsophus aequinoctialis* L., a predator of mole cricket eggs, has not yet been released in Florida, but may be released in the future (personal communication from Dr. J.H. Frank, University of Florida).

The tachinid fly *Ormia depleta* (Wiedemann) is an obligate parasitoid of *Scapteriscus* mole crickets. Female flies are phonotactic to the call of the male *Scapteriscus* spp. crickets (Fowler 1987, Fowler and Garcia 1987, Walker et al. 1996).

Phonotaxis is an uncommon host-locating method known in only one other dipteran, *Colcondamyia auditrix* Shewell, a sarcophagid parasitoid of cicadas (Soper et al. 1976). *Ormia* spp. are nocturnal and crepuscular, and collection can only be done reliably with sound emitter traps that mimic the call of the host. *Ormia depleta* was originally brought from Piracicaba, Brazil, to be used as a biocontrol agent against *Scapteriscus* spp. mole crickets and was first released in 1988 (Frank et al. 1996). Since then, it has become established in 38 counties in Florida, and in some it has been demonstrated to suppress mole cricket populations (Parkman et al. 1996). This original strain seemed to have a northern limitation to its establishment around 28° N latitude (Walker et al. 1996). Because the strain was collected near Piracicaba, Estado de São Paulo, around 23° S latitude (Piracicaba strain), it was speculated that flies collected from more temperate latitudes would be more cold-hardy and able to establish farther north in the United States. Dr. Howard Frank returned to Brazil in 1999, and collected a new strain of flies near Osório in Estado do Rio Grande do Sul, approximately 30° S latitude (Osório strain) (Frank 2002). The Osório strain was laboratory-reared and in 2000-2001, several releases were made in North Carolina, Georgia, Texas, and Louisiana. Adequate trapping and monitoring of flies in these locations have not yet occurred to determine whether the Osório strain has established (Frank 2002).

Flies in the family Tachinidae are all parasitoids of other arthropods, almost exclusively other insects (Cantrell and Crosskey 1989, Andersen 1996). In the more primitive tachinid species, eggs are deposited directly on the host, whereas other species deposit microtype eggs on their host's food plant, inject eggs into the host's body wall, or drop newly hatched larvae on or near the host (Cantrell and Crosskey 1989). The most

common hosts are the larvae of Lepidoptera and Coleoptera, but Hymenoptera, Hemiptera, Orthoptera, Dermaptera, other Diptera and, in some species, centipedes and isopods are hosts (Cantrell and Crosskey 1989, Andersen 1996). Tachinids are among the most important natural regulators of insect populations (Oesterbroek 1998). Few tachinids are known to be species-specific in their choice of host, with some species attacking hosts from three orders of insects (Cantrell and Crosskey 1989). Their success as biocontrol agents has been mixed, with many attempts failing (Cantrell and Crosskey 1989). Adult tachinid flies, in general, are known to feed at flowers and do not require any significant source of protein (Andersen 1996). They are very active and require a daily source of sugar from plant nectar, homopteran honeydew, or sap from tree wounds (Oesterbroek 1998).

Wineriter and Walker (1990) developed the original rearing protocol for *O. depleta*. This is the protocol used throughout this dissertation unless specified otherwise. This protocol requires that gravid female flies be sacrificed and the oviducts removed. The oviducts are then placed on moist filter paper and gently torn open, releasing the planidia. Three fully developed planidia are then placed behind the posterior margin of the pronotum of the host. The hosts (usually *S. abbreviatus*) are then placed in individual vials of moist sand while the larvae develop. Both male and female mole crickets are used. Twelve days after inoculation, the vials are emptied and the pupae retrieved from the sand and placed in emergence boxes. Each box contains ~2 cm moist sand and 100 pupae are placed on the sand and then covered with another ~ 5 mm layer of moist sand. Each emergence box is then placed in a large rearing cage consisting of a clear acetate tube covered on both ends with bucket lids. These cages are placed near a window that

receives indirect sunlight and the flies are allowed to emerge into the cage. Food and water are offered in Petri dishes wicked with cotton balls. After approximately four weeks in the rearing cage, gravid females are removed and sacrificed to produce the next generation. For the colonies kept at the University of Florida mole cricket rearing laboratory, two rearing cages are maintained under normal circumstances. Each cage is started with approximately 100 pupae. To obtain this number of pupae, approximately 200 *S. abbreviatus* are inoculated with three planidia each, with the expectation of obtaining two viable pupae per host (personal communication with Robert Hemenway, University of Florida).

Although this rearing protocol has been fairly successful in maintaining laboratory colonies of *O. depleta*, a new protocol was desired that would reduce the unpredictable variability in the percentage of gravid flies produced in each generation as well as allowing for smaller colonies to be maintained. These smaller colonies could be reared in environmental chambers to control the factors of photoperiod, temperature, and humidity. The number of planidia per host could be manipulated to determine the optimal use of this limiting factor in colony growth.

Because the Osório strain has been released but not yet recaptured, a method was needed to differentiate between the two strains to identify the strain of individuals recaptured in the future. This is especially important in identifying flies from areas that lie between the established range of the Piracicaba strain and the new release sites of the Osório strain.

In addition, there is some demand for a method to locally augment populations of *O. depleta* in areas where a greater level of mole cricket control is desired, such as golf

courses and bahiagrass pastures. The use of a plant/aphid banker system for the production of honeydew and subsequent attraction of *O. depleta* was investigated to determine whether the honeydew produced by this system is an adequate food source for the flies.

CHAPTER 2
VARIATION OF PARAMETERS FOR REARING *ORMIA DEPLETA* (DIPTERA: TACHINIDAE) AND INDUCING DIAPAUSE

Introduction

The protocol for rearing *O. depleta* set by Wineriter and Walker (1990) has been successful in keeping colonies at the University of Florida's mole cricket rearing laboratory almost continuously since 1988. The greatest limitation of this method is primarily in the large number of flies required per cage in order to produce gravid females. Many advantages would be gained if a method for rearing *O. depleta* in smaller colonies were to be devised.

Although the Piracicaba strain of *O. depleta* has become well established throughout southern Florida and somewhat established in central Florida, its range falls far short of that of its host (Frank et al. 1996). The releases of the Osório strain may increase the fly's range, but this has not yet been demonstrated (Frank 2002). Augmentative releases may be a possible solution, but *O. depleta* is a very difficult organism to reliably rear under the protocol set by Wineriter and Walker (1990). The populations show a great deal of instability and reproductive success is unpredictable. In an attempt to control some of the unpredictability of laboratory colonies, a revised protocol was tested to rear flies under the controlled conditions of an environmental chamber. The effects that had the greatest probability of affecting the outcome of the colonies (humidity, temperature, and photoperiod) were tested at two levels each. Natural sunlight, one of the factors long thought to be necessary for *O. depleta* to mate

successfully (Wineriter and Walker 1990), was not investigated. Dependence on natural sunlight introduces another uncontrollable variable and other research has proved that *Ormia depleta* can reproduce successfully in the absence of natural light (Welch 2000). Perhaps the most compelling reason for a new rearing method is to maintain smaller colonies. The current method requires at least 100 pupae to be used in each large (30 cm diameter × 60 cm tall, approximately 35 liter) cage (Figure 1). Maintaining fewer flies in smaller cages would facilitate experimentation and reduce the risk to the entire laboratory colony, should one colony fail. This experiment was designed to determine the levels of temperature, humidity, and photoperiod that would be most conducive to rearing *O. depleta* in small colonies in environmental chambers.

Many homopterans, including aphids, feed on the liquids they extract from plants by piercing the phloem tissues with their stylets (Owen 1978). The result is a diet very high in sugars, and excess sugars are excreted as honeydew, which is used as a food source for many organisms such as fungi, bacteria, bees, moths, ants and flies, including *O. depleta* (Owen 1978, Welch 2000). Most of the sugars found in homopteran honeydew are also occur in the saps of the plants on which they feed, such as fructose, glucose and sucrose (Percival 1965). Melezitose (α-D-glucose-[1→3]-β-D-fructose-[2→1]- α-D-glucose) is a trisaccharide found in homopteran honeydew, but not in plants (Petelle 1980). The enzyme which makes melezitose has been isolated from the midgut of aphids and shown to produce melezitose, fructose, and glucose from sucrose (Petelle 1980).

It is not fully understood why homopteran insects produce melezitose. Owen (1978) hypothesized that aphids produce honeydew because it offers them an evolutionary advantage. His theory has two requirements. The first requirement is that an

aphid and all her parthenogenically-produced offspring be considered a single evolutionary individual (Janzen 1977) such that any benefit provided to offspring can be considered benefit to self. The second, and most crucial to his argument, is that melezitose must be a sugar source which is not as widely usable to other organisms as other sugars, but highly usable to nitrogen-fixing bacteria. Aphids that produced melezitose-heavy honeydew would be benefiting their host plants by providing a nitrate source and, therefore, benefiting their offspring and, from the evolutionary individual standpoint, themselves. This theory was refuted by Petelle (1980), who found that fructose was nine times better than melezitose as a carbohydrate source for nitrogen-fixing bacteria. Instead, he postulated that, because the enzymatic action which produces melezitose prevents sucrose from breaking down into fructose and glucose and in the process ties up two molecules of glucose and one of fructose, the production of melezitose reduces the concentration of monosaccharides in the midgut of the insect. This reduction in concentration would be beneficial to the insect in that it would reduce the uptake of these sugars. Too much sugar uptake would increase the osmotic pressure and require the insect to ingest a large amount of water.

The question of how melezitose influences the longevity of *O. depleta* has been investigated (Welch 2000). It was found to be a suitable diet, indicating that *O. depleta* has the necessary enzymes to hydrolyze melezitose, but it did not perform better than a diet of hummingbird feeder nectar containing no melezitose. Although this may provide some insight into the importance of melezitose in the diet of *O. depleta*, for the purpose of maintaining laboratory colonies, longevity is not as important as the ability to produce

offspring. An experiment was designed to determine the influence of melezitose on the fecundity of mated female *O. depleta.*

Materials and Methods

Environmental Chamber Rearing Parameters

Scapteriscus abbreviatus were obtained from the mole cricket rearing laboratory at the University of Florida. This colony was originally collected from Broward County, Florida, and was inoculated with planidia from Osório strain *O. depleta.* Each mole cricket was inoculated by placing four planidia behind the posterior margin of the pronotum and then placing it in a vial of moist sand. After 12 days, pupae were collected from the mole cricket vials. Four small cups (approximately 10 cm diameter, 4 cm deep) were filled with approximately 2 cm damp sand, and 35 *O. depleta* pupae were placed in each cup on top of the sand with the spiracles pointing upward. The pupae were then covered with a thin layer (0.5-1.0 cm) of moist sand and each cup was placed in a small plastic rearing box (10 × 15 × 20 cm) lined with paper towel and covered with a lid (Figure 2). The lid was ventilated with a 10-cm-diameter hole covered with a fine screen mesh. A nutrient solution containing a 1:1:1 solution of sucrose, fructose, and melezitose (40g each /L) was kept in a small plastic Petri dish wicked with cotton and placed in each rearing box. Each rearing box was also provided with water in a small plastic Petri dish wicked with cotton. The nutrient solution was colored green for identification with approximately 25μL/L of food coloring (McCormick, Hunt Valley, MD). Both the nutrient solution and water were refilled as needed and replaced if mold appeared. Each box with pupae was then placed in a separate Florida Reach-In environmental chamber (Walker et al. 1993)

Figure 1: Old style rearing cages for *O. depleta*

Figure 2: Experimental style of rearing cage for *O. depleta*

set to a specific temperature, humidity and photoperiod. Each of these three variables had two levels determined by preliminary experimentation (Table 1) and was set up according to a balanced incomplete block design ($a=8$, $b=14$, $k=4$, $r=7$, $\lambda=3$). This design was necessary to accommodate all eight treatment combinations. A fully randomized model

would have been preferred, but this would require eight environmental chambers, which were not available, and enough pupae to set up eight experimental units, which were a greater number than our laboratory colony could reliably produce. The low humidity of 70% was used because, in preliminary trials, flies kept much below that range suffered high mortality. The high humidity of 85% was chosen because the Florida Reach In units do not do a good job of maintaining relative humidity higher than that amount. After 30 days in the chambers, all flies were removed and checked for gravidity. Mature planidia are easily visible through the abdomen of gravid females. The numbers of dead males, dead females, live males, live females, and gravid females were recorded. Gravid females were then sacrificed and their planidia used to inoculate mole crickets for the next generation. For statistical analysis, the percentage of gravid females was transformed using the arcsine square root transformation ($\sin^{-1}\sqrt{x}$). Both the arcsine square root of the percentage of gravid females and the mortality of the flies were analyzed by ANOVA (SAS Institute 2001).

Table 1: Levels of parameters tested and treatment numbers assigned

Treatment #	1	2	3	4	5	6	7	8
Humidity	70%	85%	85%	70%	70%	85%	70%	85%
Temperature	23 C	23 C	27 C	27 C	23 C	23 C	27 C	27 C
Photoperiod	10 hrs	14 hrs	10 hrs	10 hrs	14 hrs	10 hrs	14 hrs	14 hrs

Melezitose and Fecundity

Colonies of Osório strain *O. depleta* were obtained from the mole cricket rearing laboratory at the University of Florida. The colonies were reared on *S. abbreviatus* and

maintained following the protocols outlined previously, with the exception of the food provided. Colonies were provided a diet of either hummingbird nectar (Perky-Pet® Denver, Colorado, Instant Nectar, containing: unspecified proportions of sucrose, glucose, tartaric acid, sodium benzoate, artificial color and flavor), which had long been used as a sugar source for laboratory rearing at the University of Florida mole cricket rearing laboratory, or a solution of melezitose only (100 g/L). Both diets were provided ad lib and replaced if signs of mold appeared. When gravid females appeared (approximately 6 weeks after mole crickets were inoculated), females were sacrificed and their oviducts removed by dissection of the abdomen. The planidia were removed and used to inoculate mole crickets for the next generation. During inoculation, the number of mature planidia was counted and recorded. Only dark, well-sclerotized larvae were counted. Immature, light colored planidia and eggs were not counted. This procedure was repeated over several generations of *O. depleta* during the course of maintaining the laboratory colonies.

Results

Environmental Chamber Rearing Parameters

Determining the statistical significance of the variables temperature, humidity and photoperiod was not possible using the variable "ratio gravid" (number of gravid females / number of live females). Only 18 out of the 56 trials produced gravid females. The large number of zeros shifted the distribution so that parametric statistics were not meaningful. By changing the unit of measurement from treatments to individual flies, it was possible to produce a binomial distribution of female flies that survived the duration of the treatment and indicate whether or not each one became gravid. Using the arcsine square root transformation, the ANOVA results indicated that the lower temperature (23°C)

produced a higher percentage of gravid females ($F = 9.40$, $P = 0.004$) and that humidity was marginally significant ($F = 4.05$, $P = 0.052$), with the lower humidity (70% RH) producing more gravid females. Photoperiod was not significant ($P > 0.05$) nor were any of the two-way or three-way interactions (Table 2). The analysis of these data remains somewhat suspect, however, due to the fact that blocks 3, 10 and 12 failed to produce any gravid females. Because of this, the statistical software was unable to converge on a likelihood confidence interval, so some observations were eliminated from the analysis (SAS Institute 2001). Both the lower temperature and the lower humidity produced a larger percentage of gravid females (Table 3).

Table 2: Percentage of females gravid – arcsine square root transformation F-values and p-values for the main factors and interactions

Block	0.54	0.884
Temperature	9.40	0.004
Humidity	4.05	0.052
Photoperiod	2.56	0.119
Temperature x Humidity	1.60	0.214
Temperature x Photoperiod	0.75	0.394
Humidity x Photoperiod	0.01	0.994
Temperature x Humidity x Photoperiod	1.01	0.323

Table 3: Percentage of females becoming gravid within each treatment combination.

	23 C		27 C	
	10 Hours	14 Hours	10 Hours	14 Hours
70% RH	15.98%	17.92%	3.90%	3.57%
85% RH	18.79%	1.10%	0.00%	0.00%

An ANOVA analysis of the mortality of the flies showed the treatment effect to be highly significant ($F = 3.67$, $P < 0.003$). The percentage mortality for the eight treatments ranged from 30.4% to 72.6% (Table 4). The higher temperature and longer photoperiod produced higher mortality, whereas humidity and all two and three way interactions were not significant (Table 5). The block effect was not significant ($F = 1.51$, $P > 0.15$).

Table 4: The effect if temperature, humidity, and photoperiod on the percentage of mortality of *O. depleta*

	23 C		27 C	
	10 Hours	14 Hours	10 Hours	14 Hours
70 % RH	39.1%	41.3%	44.7%	67.3%
85% RH	30.4%	44.0%	55.6%	72.6%

Table 5: Mortality F-values and p-values for the main factors and interactions

Source	*F* -value	*P* < *F*
Block	1.51	0.156
Temperature	13.87	0.0005
Humidity	0.65	0.424
Photoperiod	11.24	0.002
Temperature x Humidity	1.46	0.232
Temperature x Photoperiod	0.77	0.386
Humidity x Photoperiod	0.43	0.514
Temperature x Humidity x Photoperiod	2.85	0.098

Melezitose and Fecundity

The planidia of 11 melezitose-fed flies and 12 non-melezitose-fed flies were counted. The mean number of planidia for melezitose-fed females was 248 ± 73 (SD) with a range of 121-392. For non-melezitose fed females, the mean was 232 ± 71 (SD)

with a range of 117-341. A t-test showed no difference between these two treatments (P = 0.61).

Discussion

The rearing of *O. depleta* inside environmental chambers was only somewhat successful. For some generations, more flies became gravid in the four environmental chambers than in the two large laboratory colonies. However, as previously stated, there were several generations that did not produce any gravid females in the environmental chambers. No tests were conducted to determine whether the colonies inside the chambers would be more successful if maintained as large colonies versus the small colonies used in this experiment.

Temperature was the factor of greatest significance. Although the flies can develop normally at temperatures above 30° C (Cabrera 2000), mold appeared more often in the chambers kept at 27° C versus those kept at 23° C. Food containers had to be changed very frequently and fungi were visible growing on the paper towel lining the bottom of the cage on several occasions. The increase in mortality from 23° C to 27° C (38.7% vs. 60.1%) could have also been due in part to problems caused by fungal growth. Fungi have been known to cause high mortality in laboratory colonies of *Drosophila hydei* Sturtevant and *D. melanogaster* Meigen (Hodge and Mitchell 1997).

It was surprising that humidity was not a more significant factor in the successful rearing of *O. depleta* than the data show (P = 0.052). It may have been discounted somewhat in this experiment due to the fairly narrow range of humidities used. The lower humidity level most likely was more successful again because of reduced fungal growth. There was a greater incidence of fungi in the high humidity chambers over the low

humidity chambers. The mortality between the two levels of humidity was essentially the same. Humidity is often a major source of mortality in rearing insects (Tsitsipis 1980, Leatemia et al. 1995); this further supports the hypothesis that the range of humidity tested here was too narrow to show the real significance of this factor.

Photoperiod was tested due to the traditional thinking that sunlight was in some way necessary for *O. depleta* to mate successfully. The production of gravid females within the environmental chambers showed that sunlight is not a necessity for *O. depleta* to reproduce; however, it would be irresponsible to discount that the regular laboratory colonies did, for some generations, produce more gravid females than those in the environmental chambers. Until specific testing has been conducted, no changes would be suggested to that particular practice. It was unexpected to find such a drastic difference between the mortality of the flies kept on the two levels of photoperiod. The higher mortality caused by the longer photoperiod may have been due to these flies having to go longer periods without eating, as the flies do not often appear to feed actively during the daylight hours.

Because some generations did not produce any gravid females in the small colonies in the environmental chambers, it cannot be recommended that this method be adopted for regular laboratory use. Future research in this area may investigate how larger colonies of flies, such as those currently used in laboratory rearing of *O. depleta*, are able to produce gravid females in environmental chambers. The most likely parameters to be successful would be in the range of the lower temperature and lower humidity used in this experiment.

Previous research has shown that melezitose does not increase or decrease the survival of *O. depleta* compared to other carbohydrate diets (Welch 2000). In the ichneumonid wasp *Bathyplectes curculionis* Thomson, longevity was significantly reduced when fed a diet of melezitose versus other sugars (Jacob and Evans 2004). This indicates that *O. depleta* does have some benefit from melezitose. There is, however, no evidence that it is important for reproductive success. Comparable fecundities were produced by both a diet of melezitose and a non-melezitose diet supports the hypothesis that *O. depleta* is an opportunistic feeder rather than an obligate honeydew feeder. It would seem that an obligate honeydew feeder would produce fewer offspring from being fed only a melezitose-free diet. This study showed no such reduction. Whether melezitose might have an effect on the fecundity of *O. depleta* when combined with other sugars remains to be tested. This study does support the hypothesis that *O. depleta* has the necessary enzymes to hydrolyze melezitose.

Previous research showed that sucrose is quickly broken down in the crop of the fly, but melezitose is hydrolyzed very slowly (Welch 2000). Burkett (1998) found similar results in carbohydrate hydrolysis in mosquitoes. The likely location for melezitose hydrolysis is the midgut. There may be some advantage to feeding on melezitose in that it would be absorbed more slowly than other sugars, prolonging the time over which energy could be derived from the food. This may make it less necessary to store energy in the form of trehalose, fats or glycerol, which require energy to synthesize. Another possible advantage of feeding on melezitose lies in its availability. The visual and/or olfactory attractants associated with honeydew have not been investigated as they apply to *O. depleta*. Whether these flies search out honeydew or come upon it randomly is unknown.

But once found, it is an easy food source to access. No specialized mouthparts are required to reach the source, in contrast to many floral nectars, nor is it necessary to pierce the skin of fruits or other plant tissues. The apparently non-specialized mouthparts of *O. depleta* could be the result of two possibilities. Either they are truly opportunistic and feed on whatever carbohydrate sources they are able to locate, including honeydew, and have never evolved any type of specialized feeding apparatus, or their mouthparts are specialized and have evolved to feed primarily on honeydew. A good test for this would be to determine whether *O. depleta* have other behavioral or physiological traits which show a specialization towards honeydew feeding. One such characteristic would be the flies' ability to locate honeydew deposits. If *O. depleta* has evolved the ability to locate honeydew by olfactory or visual cues, then it is likely that it could be considered more of a specialist feeder on honeydew than if it were to only find it by chance. The high percentage of field-caught *O. depleta* which had fed on honeydew, as indicated by the presence of melezitose in the crop (Welch 2000), seems to support the hypothesis that they are not merely encountering honeydew randomly. Future research into the attractant properties of honeydew, honeydew constituents, and plant/aphid systems to *O. depleta* would be very useful both for determining the true specialization of this species as well as for making banker plant recommendations for localized augmentation of fly populations.

CHAPTER 3
INTRASPECIFIC COMPETITION FOR RESOURCES BY *ORMIA DEPLETA* (DIPTERA: TACHINIDAE) LARVAE

Introduction

Ormia depleta can be a difficult organism to maintain in a lab colony. One of the factors that makes them difficult lies in the variable and generally low proportion of gravid females obtained under the current lab rearing protocol. A colony of 100 individuals may in one generation produce 20 gravid females and in the next only one or two or even zero. Therefore, it is necessary to determine the best way to use the number of planidia available in any one generation to produce the maximum number of healthy pupae to start the next generation. This must also be balanced with the expense of rearing the mole cricket hosts, which are very labor intensive to maintain. Current laboratory protocol dictates that three planidia be placed under the posterior margin of the pronotum of each *Scapteriscus* host when inoculating by hand (Personal communication with Robert Hemenway, University of Florida). If there is competition among the larvae for resources within the host, fewer planidia per host may increase the chances of survival for those planidia and may produce larger pupae. This would, however, require more hosts to produce enough pupae to maintain the colony. Inoculating hosts with more planidia may increase the number of pupae and reduce the cost associated with host rearing, but superparasitoidism should be avoided as well as the production of pupae and adults with reduced fitness.

The effect of superparasitoidism on the developing larvae of many insect species has been studied extensively. Superparasitoidism has been shown to increase larval development time in *Cotesia glomerata* (L.) (Gu et al. 2003), *Microplitis croceipes* (Cresson) (Eller et al. 1990), and *Cotesia flavipes* Cameron (Potting et al. 1997). However, Bai and Mackauer (1992) found no increase in the larval development time of *Aphidius ervi* Haliday when subjected to superparasitoidism. In some species, it has been shown to decrease survivorship (Vinson and Sroka 1978, Gu et al. 2003) but not in other species (Harvey et al. 1993). The offspring of the ichneumonid wasp *Venturia canescens* (Gravenhorst) were smaller under the condition of superparasitoidism (Harvey et al. 1993), but the wasp *A. ervi* actually increased in size (Bai and Mackauer 1992). The most common effects of superparasitoidism apparently are an increase in the number of brood produced, but with an overall reduction in the fitness of those offspring (van Dijken and Waage 1987, Vet et al. 1994).

Previous research with *O. depleta* showed that there was no relationship between the number of planidia used to inoculate the host and the number of pupae produced (Fowler 1988), but preliminary research done here showed that higher numbers of pupae could be produced than previously recorded. Additionally, Fowler and Martini (1993) found a weak correlation between host size used and the weights of the flies produced. In the present experiment, this relationship was also examined to determine whether host weight is an important factor in determining which hosts should be used. The goal of this experiment was to determine if an increase in the number of pupae produced per host could be achieved without sacrificing the survivability or vigor of the larvae. In addition to varying the number of planidia applied to each host, the weights of the host mole

crickets were measured during inoculation to see whether larger hosts could provision more parasitoids. These factors of host mole cricket weight and number of planidia used to inoculate the host were examined to determine their effect on the number of pupae produced, the mean weight of those pupae, and the survivability of the larvae to the pupal stage.

Materials and Methods

During the maintenance of the laboratory colony of *O. depleta*, *S. abbreviatus* from the University of Florida mole cricket rearing lab were individually weighed and inoculated with varying numbers of *O. depleta* planidia. The weights of the hosts ranged from 0.54-1.59 g and the weights of the hosts were not considered in determining the number of planidia used to inoculate each individual. The number of planidia per host ranged from 2-8, with most of the mole crickets being inoculated with either 3, 4, or 5 planidia. These numbers were favored because they are the numbers most frequently used in the routine maintenance of the colony. The number of planidia placed on each host was randomly determined. The numbers of mole crickets inoculated with 2, 3, 4, 5, 6, 7, and 8 planidia were 12, 108, 110, 52, 43, 32, and 11, respectively. Each mole cricket was then returned to an individual 20 dram plastic vial filled with moist sand, and the larvae were allowed to develop for 12 days at a room temperature of ~26°C. At that time, the pupae were collected and weighed. Statistical analysis was performed using the general linear model procedure (SAS Institute 2001). Regression analysis was used to determine how differing numbers of planidia affected the number of pupae produced, the mean pupal, and the survivability of the planidia. Additionally, the weights of the host mole crickets were analyzed to determine their effect on the survivability of the planidia used. Where

applicable, the differences between the means were determined by Duncan's multiple range test (SAS Institute 2001). Regression analyses were conducted to determine the relationships between each of these factors (SAS Institute 2001).

To determine the effect that host mole cricket weight had on planidia survival, the number of pupae produced and the mean weights of those pupae, mole cricket weights were rounded up to the nearest 0.1 gram to place them into weight classes. Additionally, weight classes which had only two or fewer samples were eliminated. In this case, the smallest weight class, 0.70 grams (n = 2) and the two largest weight classes, 1.5 grams (n = 2) and 1.6 grams (n = 2) were eliminated from the statistical analysis. The survival of the planidia on hosts in the remaining weight classes were analyzed by ANOVA (SAS Institute 2001).

Results

The mean number of the pupae produced relative to the number of planidia used can be seen in Figure 3. There is an increase in the number of pupae produced as the number of planidia increases ($F = 15.77$, $P < 0.0001$) and significant differences between the means of the treatments. The regression analysis (Figure 4) supports this trend and indicates an increase of 0.41 pupae for each increase in planidia ($F = 83.77$; $P < 0.0001$; $r^2 = 0.19$).

Figure 5 shows the survival of planidia grouped by the number of planidia placed on each host. ANOVA is significant for the model (F=2.57, $P < 0.02$). Figure 6 is the regression analysis of the same data set ($F = 9.16$; $P < 0.002$; $r^2 = 0.03$), indicating an approximate 3% reduction in survival for each increase in the level of planidia density.

The analysis of the number of planidia used as it affected the mean weight of the pupae produced was found to be nearly significant by ANOVA (F=2.06, $P = 0.057$).

There was some significance among the means as indicated by the letters over the bars in Figure 7. The regression analysis for the mean weights of pupae produced is in Figure 8 ($F = 8.33$; $P < 0.004$; $r^2 = 0.02$) and indicate a reduction in the mean weight of the pupae of 2.2 mg for each additional planidia.

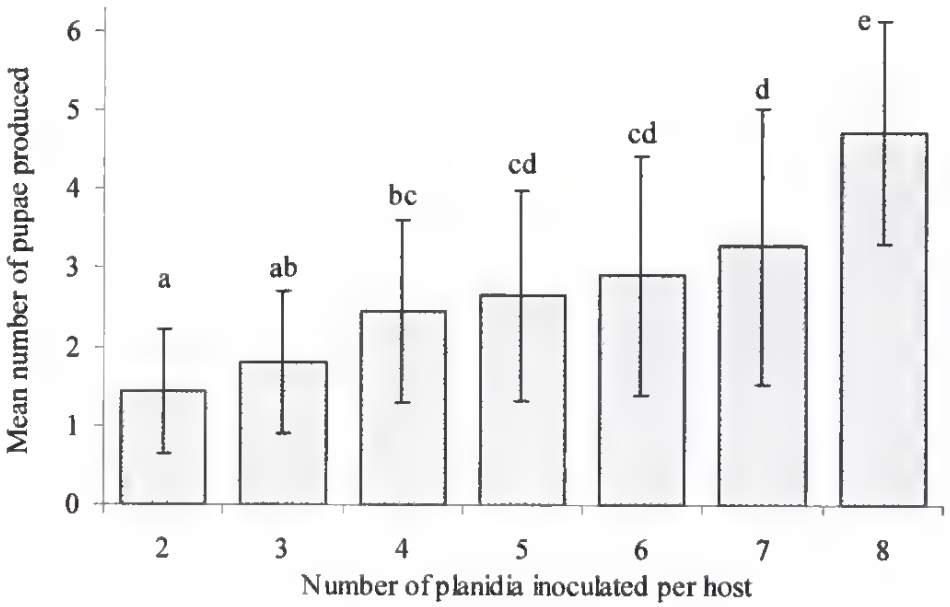

Figure 3: The effect of planidia density used to inoculate mole crickets on the number of pupae produced (error bars indicate standard deviation, significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$)

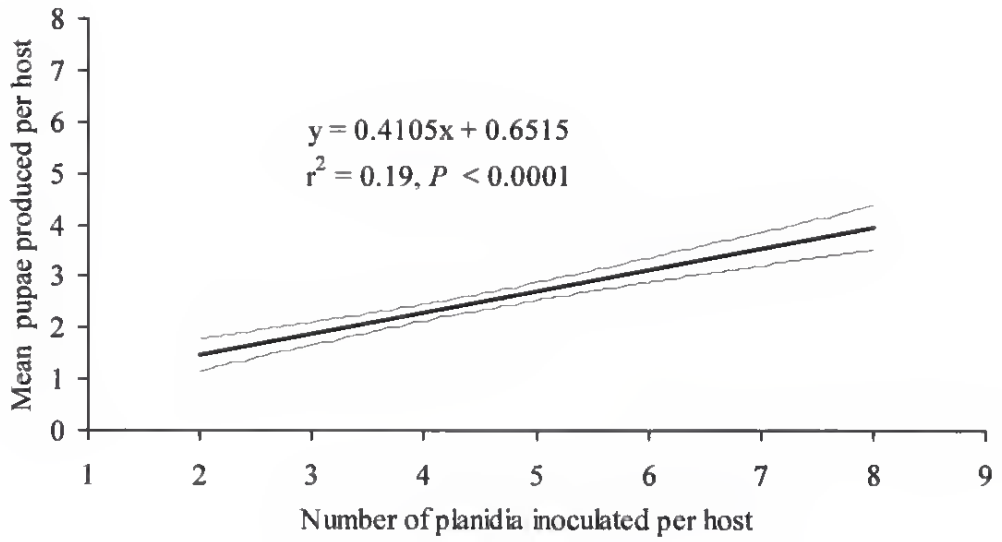

Figure 4: The effect of number of planidia used to inoculate mole crickets on the number of pupae produced – regression analysis with 95% confidence bands

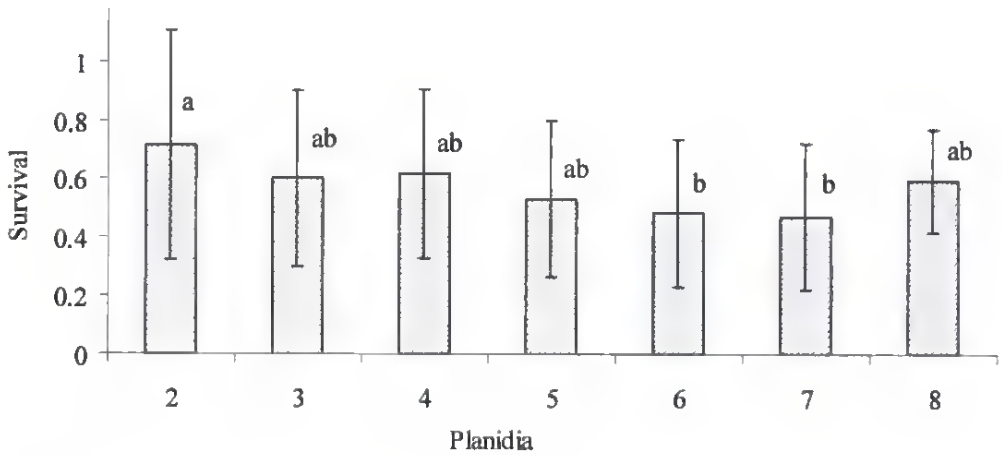

Figure 5: The effect of number of planidia used to inoculate mole crickets on the survival rate of the larvae to the pupal stage (error bars indicate standard deviation, significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$)

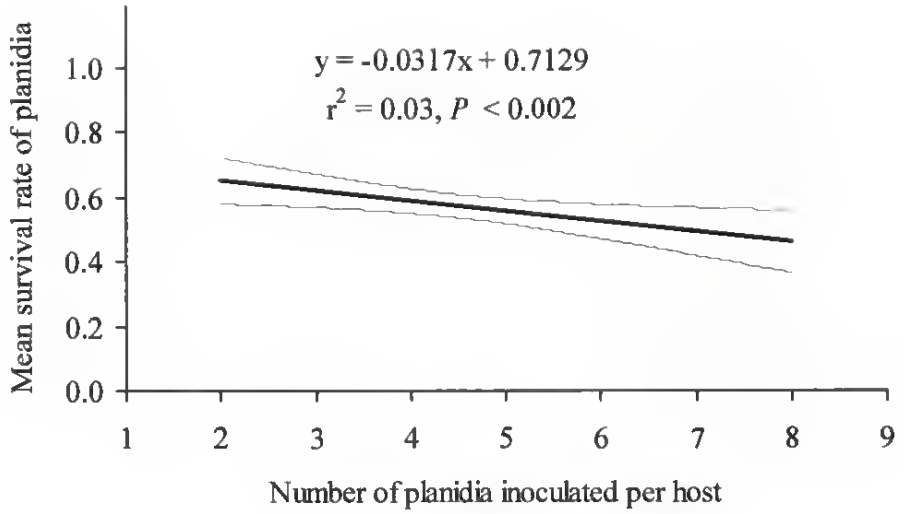

Figure 6: The effect of number of planidia used to inoculate mole crickets on the survival rate of the larvae to the pupal stage – regression analysis with 95% confidence bands

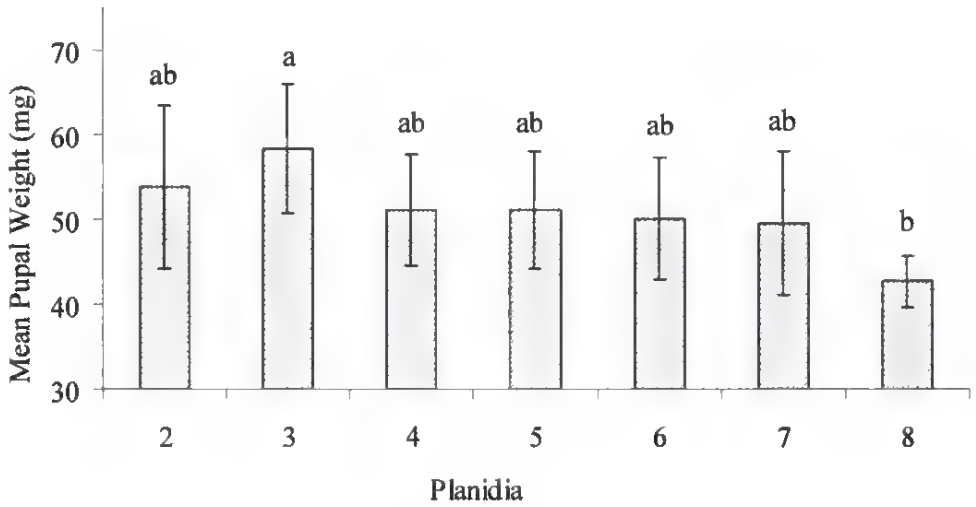

Figure 7: The effect of number of planidia used to inoculate mole crickets on the mean weight of the pupae produced (error bars indicate standard deviation, significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$)

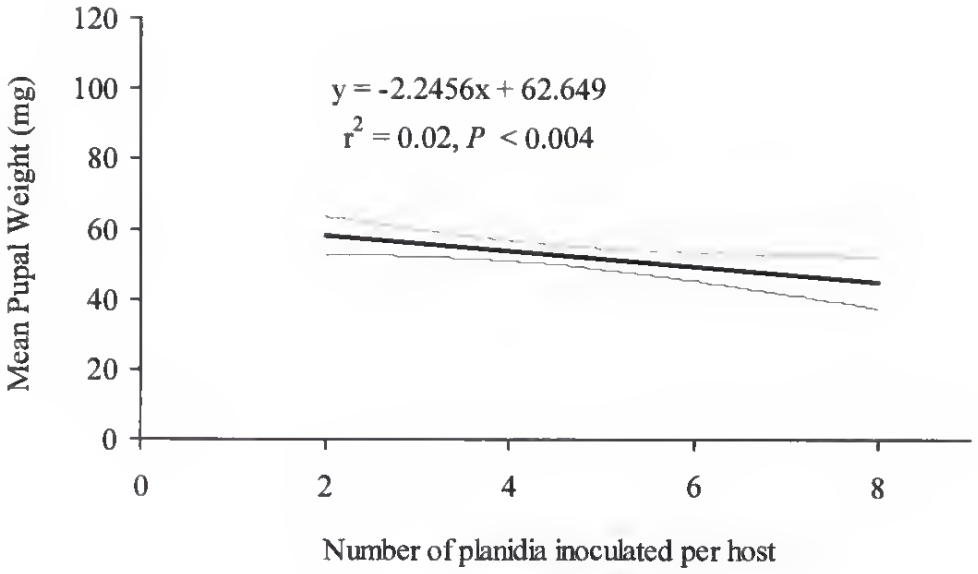

Figure 8: The effect of number of planidia used to inoculate mole crickets on the mean weight of the pupae produced – regression analysis with 95% confidence bands

The effect that host mole cricket weight had on the number of pupae produced was not found to be significant when analyzed by ANOVA ($F = 1.06$, $P = 0.39$). The effect that host mole cricket weight had on the survivability of the larvae was found to be marginally significant ($F = 2.12$, $P = 0.0505$). The effect that host mole cricket weight had on the mean weight of the pupae produced was highly significant ($F = 3.49$, $P < 0.002$) (Figure 9). The regression analysis can be seen in Figure 10 ($F = 20.62$; $P < 0.0001$; $r^2 = 0.05$).

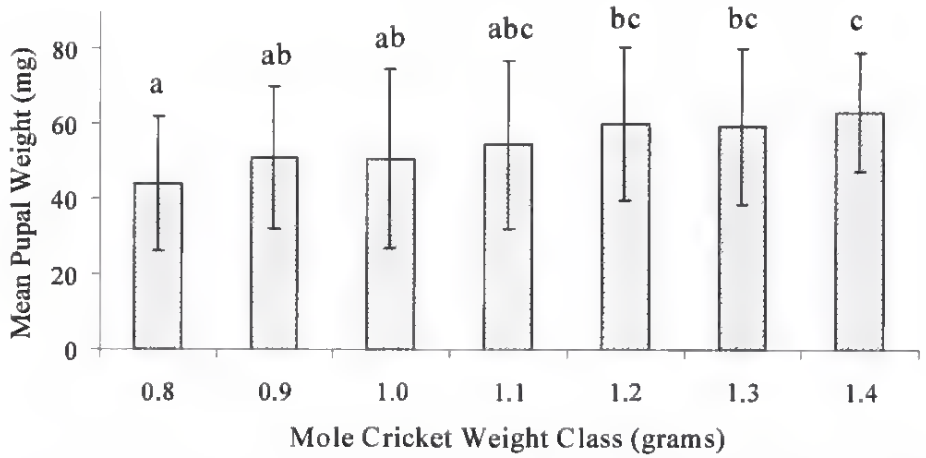

Figure 9: The effect of host cricket weight on mean pupal weight (error bars indicate standard deviation, significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$)

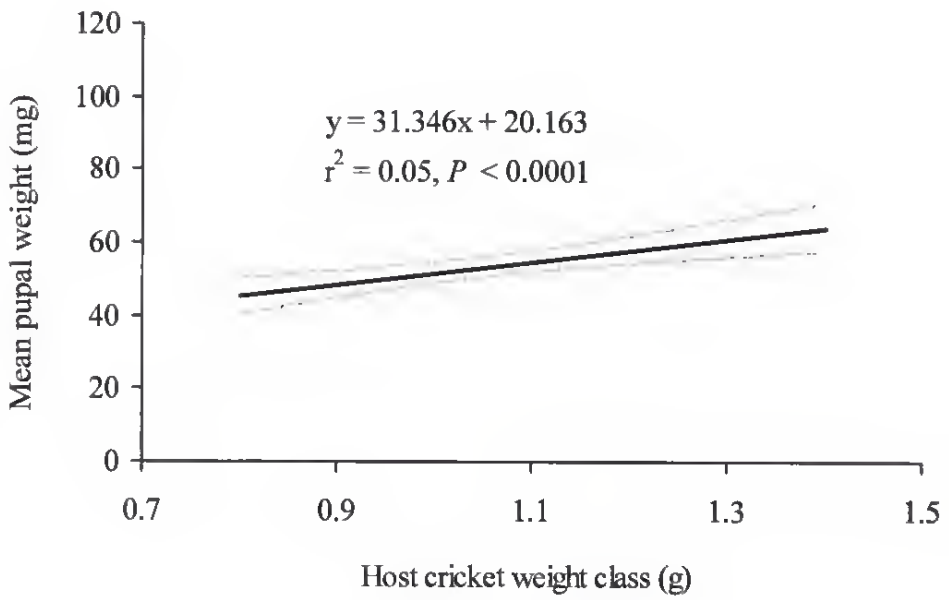

Figure 10: The effect of host cricket weight class on the mean weight of the pupae produced – regression analysis with 95% confidence bands

Discussion

The number of planidia used to inoculate host mole crickets as well as the weight of those mole crickets are important factors to the rearing of *O. depleta* in the laboratory. Although these data do not clearly dictate a specific protocol that should be used, they do

provide a framework that would allow for anyone rearing *O. depleta* to structure an inoculation protocol specific to their needs. At times when large numbers of planidia are available but few hosts are available, the data suggest that inoculating mole crickets with more planidia would increase the production of pupae. Too many, however, would result in reduced pupal size. At times when fewer planidia are available and maximum survivability is required, inoculating two or three planidia per host would be more effective. Alternatively, if larger pupae are desired, reducing the number of planidia per host along with using larger hosts would achieve the desired goal. Therefore, the current method of inoculating three planidia per host is less efficient than inoculating four or five, since there is no significant reduction in pupal size, but there is a significant increase in the number of pupae produced. The reduction in size that results from the use of eight planidia, or possibly more, would likely be detrimental to the colony of flies. Some preliminary data (Appendix A) has shown that pupae less than 40 mg have reduced survivorship to the adult stage. This reduction is greater than the increase in number that is gained when going from 6 or 7 planidia to 8 planidia. Furthermore, these data only show a reduction in the emergence rate of the pupae, they do not indicate other negative factors which may be associated with reduced size. Future research may be needed to determine whether these smaller individuals show any reduction in longevity, ability to mate, or in fecundity as well as how the reduction in size of a generation may affect the size or fitness of future generations of flies.

According to the University of Florida's Integrated Pest Management Website (http://ipm.ifas.ufl.edu/extension-resources/glossaries/glossary.htm), the definition of superparasitoidism is

> the situation in which more individuals of a parasitoid species develop in a host than can obtain adequate resources to complete their development. Females of some parasitoids may lay more than one egg in or on a host, resulting in superparasitoidism, although the behavior of females tends to avoid this condition by discriminating against already-parasitoidized hosts. In fact, five conditions can be distinguished (a) only one parasitoid exists within the host; (b) there is more than one parasitoid within the host, but all survive and produce adults of normal size [this is not superparasitoidism]; (c) there is more than one parasitoid within the host and they all survive but produce adults of subnormal size because of competition for resources [this is viewed here as superparasitoidism]; (d) there is more than one parasitoid within the host and some of them die due to competition for resources (including attack by conspecifics) [this is superparasitoidism]; and (e) there is more than one parasitoid within the host and all die because the resources are too few [this is superparasitoidism].

By this definition, the situation of *O. depleta* in this experiment qualifies as superparasitoidism only under the highest number of planidia inoculated on the host. Although some reduction in size is apparent at almost all levels above three planidia, most of the reductions are not significant.

This raises an interesting question as to the natural behavior of the flies. Under field conditions, the mean number of *O. depleta* larvae found within trapped *Scapteriscus* hosts is less than two (Amoroso 1990). It would seem likely that, due to the flies' phonotactic search method for hosts and the solitary nature of adult mole crickets, it would be to the flies' advantage to maximize the number of offspring that it would be able to produce from the seemingly limited number of hosts. But apparently, this is not the case. The closely related *O. ochracea* Bigot, a parasitoid of *Gryllus* spp. crickets, have an optimal laboratory clutch size of 4-5 larvae per host, but under field conditions only deposit 1.7 ± 1.0 S.D. larvae (Adamo et al. 1995). There must be some ecological advantage to depositing fewer larvae than what appears to be the optimal number.

It may be that *O. depleta* does not suffer from any shortage of hosts. Mole crickets are certainly abundant and calling during certain times of the year, but at other

times seemingly unavailable. *Ormia depleta* may be able to find non-calling mole crickets in other ways, or there may be alternative hosts (this is discussed more in Chapter 4). Adamo et al. (1995) concluded that host availability was not a likely factor in determining the number of larvae deposited on hosts by *O. ochracea*. Another possibility is that *O. depleta* is responding to a factor in the field that is greatly reduced in the laboratory, the mortality of the hosts. Under laboratory conditions, mole crickets suffer little disease and no predation. It may be that in the field, the higher mortality of the hosts would make it advantageous to partake in bet hedging and spread offspring out over many hosts so that the loss of one host has a lesser effect on the total number of offspring. This hypothesis is somewhat strengthened by the fact that *O. depleta* does not deposit eggs, but planidia larvae, so the female's investment in parasitoidizing a host is already greater than that of an egg layer. Another laboratory factor that should be considered is hand-inoculating. The mole crickets that are hand-inoculated are unable to protect themselves in any way and have no opportunity to use whatever natural defenses they may have available in the field. It may be that, although it would be advantageous for *O. depleta* to parasitoidize hosts with a greater number of planidia, the natural behavioral defenses of *Scapteriscus* mole crickets prevent it, whether those defenses involve brushing off planidia or simply retreating underground when the presence of *O. depleta* is detected. This type of grooming has been observed in *Gryllus* spp. crickets after an encounter with *O. ochracea* (Adamo et al. 1995).

One possibility that has been suggested is that *O. depleta* are parasitoidizing hosts with higher numbers of planidia in the field, but we are unaware of it. The trapping methods used to determine parasitoid levels of mole crickets in the field are usually

sound traps, which require the mole crickets to fly into the trap, or pitfall traps, which require the mole crickets to be actively crawling on the soil surface to be captured. It may be that those mole crickets with larger parasitoid loads are under greater physical stress and are less able or likely to venture to the surface where they can be trapped. Zuk et al. (1993) found that the calling of the field cricket *Teleogryllus oceanus* (Le Guillou) was inhibited by infestation of *O. ochracea* in Hawaii. Although Zuk did not report on these crickets' ability to move, it does suggest that there is a physical liability to carrying parasitoids. Collecting mole crickets by digging and sifting soil would probably produce more accurate results than the current trapping methods, but the labor and expense involved would not justify the change in methods.

The final reason for the low numbers of larvae found in field captured hosts may be that there is a reduction in fitness caused by the high numbers of larvae used in this experiment that were not investigated. Reduced size is the easiest type of fitness reduction to observe, but many others may be at work. It may be that, due to competition, certain key resources are not available in sufficient amounts for the flies reared under superparasitoid conditions for the resulting adult flies to develop, mate, locate hosts, or reproduce properly. Many physiological deficiencies may result from superparasitoidism, and they may not be obvious either externally, or immediately (Waage and Ng 1984). These possibilities still remain for future research.

CHAPTER 4
REARING *ORMIA DEPLETA* (DIPTERA: TACHINIDAE), A PARASITOID OF MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE), ON A FACTITIOUS HOST, *ACHETA DOMESTICUS* (ORTHOPTERA: GRYLLIDAE)

Introduction

The maximum life span of adult *O. depleta* in the lab is approximately 60 days (Welch 2000), which would not allow them to survive between times of peak mole cricket calling activity, which are from February to June (Walker and Moore 2000). Therefore, some sort of diapause might be expected to allow the flies to carry over from June to the Following February. Some preliminary research was made to determine whether *O. depleta* pupae could be stimulated to diapause by exposing them to cold temperatures (0 - 5° C), but refrigeration attempts at these temperatures resulted in 100% mortality. Additionally, work done by Cabrera (2000) found that attempts to rear larvae at temperatures below 17° C resulted in the death of the larvae. Therefore, cold-induced diapause does not seem to be a probable part of *O. depleta*'s life history. Other experimentation (Appendix B) suggests that there is no photoperiod-induced diapause. The question then remains: what happens to the population of *O. depleta* during the months between the period of peak calling activity of *Scapteriscus* spp. hosts?

It is possible that *O. depleta* locate mole cricket hosts when the mole crickets are not known to call in large numbers. The flies may be able to find enough to maintain their population until the next calling season. Calling traps located at the Gulf Coast Research and Education Center in Bradenton, Florida, catch most of their *O. depleta* during the seasons that follow peak adult *Scapteriscus* flight activity, which are from March through

May and from September through November, but some mole crickets are caught during every month of the year (Walker et al. 1992a, b). Dr. Thomas Walker has also observed individuals of *S. borellii* and *S. vicinus* calling in each month of the year, albeit in much smaller numbers than in the peak seasons (Walker and Moore 2000). If only few flies were able to locate *Scapteriscus* hosts during these times, it could maintain a seed population until the next calling season.

Another possible solution is the use of alternate hosts. *Ormia depleta* is able to survive through the winter in central Florida. Specimens are collected in Bradenton, Florida, throughout the year, although the greatest numbers coincide with the peak mole cricket flight seasons (Walker et al. 1992b). Bradenton does occasionally experience cold weather and even frost. This may suggest that during these times when adult or pupal *O. depleta* would not be able to survive, that perhaps the larvae are able to survive within a host. Being within a host would provide a great deal more protection from the environment. If *O. depleta* were unable to reliably locate *Scapteriscus* hosts during these times when they are not calling, it could be possible that they were using an alternate host. This has been suggested by Fowler and Mesa (1987) with the recovery of *O. depleta* from a species of *Anurogryllus.* Additionally, Justi et al. (1988) reported that under laboratory conditions, *O. depleta* successfully developed within an unnamed *Gryllus* species. Although there is no evidence to suggest that *O. depleta* is phonotactic to any taxa other than *Scapteriscus* (Fowler and Garcia 1987), it is plausible that it may occasionally encounter enough hosts by other means to maintain a population between the calling seasons of its preferred host.

Multiple hosts among tachinids are not unusual. Some tachinids have host ranges that cover three orders of insects (Cantrell and Crosskey 1989). *Ormia depleta* is thought to be and reported as an obligate parasitoid of *Scapteriscus* mole crickets (Frank et al. 1998), and under most conditions, it probably is. The mechanism for the specificity of *O. depleta* lies in its phonotaxis. There are no reports of it being attracted to any calls other than those of *S. vicinus* and *S. borellii* (Walker et al. 1996). Under field conditions, this greatly limits the fly's ability to select new hosts. Under laboratory conditions, especially when hand-inoculating hosts, it may be possible to manipulate this characteristic. It would be advantageous to be able to rear *O. depleta* on an alternate host when *Scapteriscus* are unavailable or simply to reduce the costs associated with rearing *Scapteriscus*. The effect of rearing tachinid flies on factitious hosts has been examined many times. Baronio et al. (2002) found there to be no decrease in the pupal weights of *Pseudogonia rufifrons* Wiedemann when reared on the factitious host *Ostrinia nubalis* (Hubner) rather than on *Galleria mellonella* L., its natural host. The tachinid fly *Exorista larvarum* (L.) was found to be even more successful on the factitoius host *G. mellonella* than on its natural host, *Lymantria dispar* (L.) (Dindo et al. 1999). With this in mind, a factitious host for *O. depleta* was examined.

Acheta domesticus (L.) (the house cricket) can be reared in large groups inexpensively and would be an economical substitute for *Scapteriscus* spp. as a host in laboratory rearing, if able to support the development of *O. depleta*. Any failure of *O. depleta* to successfully develop in an *A. domesticus* host would lead to the question of why they did not develop well. Was there failure to penetrate the host? Did *A. domesticus* lack sufficient nutritional value? One of the major factors differentiating *A. domesticus*

and *Scapteriscus* spp. behaviorally is that *Scapteriscus* spp. are primarily subterranean whereas *A. domesticus* is not. Although this would not seem to have a direct effect on internally developing larvae, it would be likely to have an effect on the ability of the larvae to pupate after leaving the host. There may also be differences in the cuticles of the two crickets that could hinder the development or the emergence of the larvae. To study this, the larval behavior of *O. depleta* inside both *S. abbreviatus* and *A. domesticus* was observed.

Materials and Methods

Larval Development of *Ormia depleta* in *Acheta domesticus*

Ten adult *A. domesticus* were obtained from the colony kept at the University of Florida mole cricket rearing lab. Each cricket was inoculated with four planidia from a freshly sacrificed gravid female *O. depleta* from the Piracicaba strain laboratory colony. Inoculated crickets were placed in a 30 × 23 × 10 cm clear, plastic container with a screen ventilated top. The bottom of the container was filled with approximately 3 cm of moist, autoclaved sand. A screen of 0.6 cm galvanized mesh was suspended approximately 3 cm above the sand to keep the crickets off the sand but to allow newly emerged larvae to drop through to the sand. Cardboard tubes 5 cm in diameter were placed on the galvanized screen to provide hiding places for the crickets. Water was made available by placing a Petri dish on the galvanized screen wicked with cotton. Food was also provided (Nutrena Cricket and Earthworm Feed, Minneapolis, MN), also in a Petri dish on the screen. Food and water were available ad lib. The crickets were observed daily and their food and water changed as needed. Approximately 10 days later, when all crickets were dead and emerged larvae had pupated, the pupae were removed and their number and weight were recorded. This process was repeated for four generations of *O. depleta*.

Larval Behavior within host

Acheta domesticus and *S. abbreviatus* were obtained from the mole cricket rearing lab and inoculated with Osório strain *O. depleta* planidia. Twenty-one crickets from each species had four planidia placed under the posterior margin of the pronotum. *Acheta domesticus* were then placed in a small container containing food (Nutrena Cricket and Earthworm Feed, Minneapolis, MN) and water in a Petri dish wicked with cotton. *Scapteriscus* spp. were returned to their vials of sand. The planidia were then allowed to develop in their hosts. Three crickets were sacrificed at each of seven 24-hour intervals, dissected, and the larvae searched for. When found, the size, number, location, and apparent tissues being fed upon were all noted. A t-test was used to compare the mean pupal weights of pupae reared on *A. domesticus* to those reared on *S. abbreviatus* (SAS Institute 2001).

Results

Larval Development of *Ormia depleta* in *Acheta domesticus*

Acheta domesticus was able to act as a factitious host for *O. depleta* in that some of the larvae were able to survive to the pupal stage. However, the survivorship of the planidia was only 29% versus 57% in *S. abbreviatus* (as determined in Chapter 3). Additionally, the weight of the pupae produced was significantly less than those produced in *S. abbreviatus*. The mean pupal weight when reared in *A. domesticus* was 39.85 mg ± 1.53 (SE), which was significantly smaller than those in *S. abbreviatus* , where the mean pupal weight was 54.60 mg ± 0.50 (SE) ($P < 0.001$; df = 950).

Larval Behavior within host

Twenty-one *A. domesticus* and twenty-one *S. abbr*eviatus were examined in this experiment. At least one planidium was found in each host species for each day of this experiment. The mean number of planidia found and the mean length of those planidia are shown in Table 6.

Table 6: Mean number and length of *O. depleta* larvae found in *S. abbreviatus* and *A. domesticus* hosts

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
S. abbreviatus							
Mean number of larvae found	0.67	1.33	2.67	3.00	2.33	2.33	2.33
Mean length (mm)	0.62	0.72	2.29	4.86	9.78	11.87	12.69
A. domesticus							
Mean number of larvae found	0.33	1.00	1.00	1.67	2.33	1.67	2.00
Mean length (mm)	0.58	0.64	0.85	1.44	4.57	7.25	8.94

The following is a timeline of the activity of the larvae inside each host.

Scapteriscus abbreviatus-

Day 1 (24 hours after inoculation)- A few planidia were dead on the exterior of the host. The larvae that succeeded in penetrating the host are very difficult to find – only two were found among the three hosts dissected. Those that were found were in the fatty tissues just below the area where they had been placed, apparently feeding on those tissues. (Figure 11).

Day 2- Still some dead planidia are visible on the exterior of the host. Larvae are easier to find, though still small. The larvae are now distended and almost transparent. Some of the larvae are still found in the fatty tissues under the pronotum while a few have moved to a more posterior and lateral position. The farthermost observed was located along the left side of the abdomen, just posterior to the metacoxa (Figure 12).

Day 3 – All larvae found were in the abdomen of the host. They were found in clusters on either side in the area near the gonads. They appeared to be feeding in fat tissue and/or reproductive organs (Figure 13).

Day 4 – Larvae are still in the gonadal region. The ovaries/testes are no longer visible in large amounts. The larvae are now posteriorly attached to the host exoskeleton. The alimentary canal of the host, though in reach of the larvae, does not appear to be damaged (Figure 14).

Day 5 – The attachment points of the larvae are now visible on the exterior of the host and appear as small, dark brown spots (Figure 15). The fat bodies within the reach of the attached larvae are now greatly reduced or gone. The alimentary canal still appears to be undamaged (Figure 16).

Day 6 – Hosts are starting to act very sluggish and showing signs of dying. The larvae now occupy approximately half of the area within the hosts' abdomens (Figure 17). The heads of the larvae can now reach into the thorax and they are apparently feeding there. The inside of the abdomen now looks very "dry" as there are no remaining fat reserves, no gonads, and no hemolymph in any large amount. The alimentary canal, though well within reach of the larvae, shows no signs of damage. The attachment points of the larvae are now visible from the outside of the host and appear as small brown patches, approximately twice the size of the hosts' spiracles. On the inside, the points of attachment seem to open into small scleritized cones that appear to be attached to the posterior ends of the larvae by what appear to be the old exuviae (Figure 18).

Day 7 – Larvae are much less active in the host. Some still appear to be feeding on the remaining tissues in the thorax of the host, others appear to be at rest. The alimentary canal of the host remains undamaged (Figure 19).

Figure 11: Larva of *O. depleta* in *S. abbreviatus* host one day after inoculation

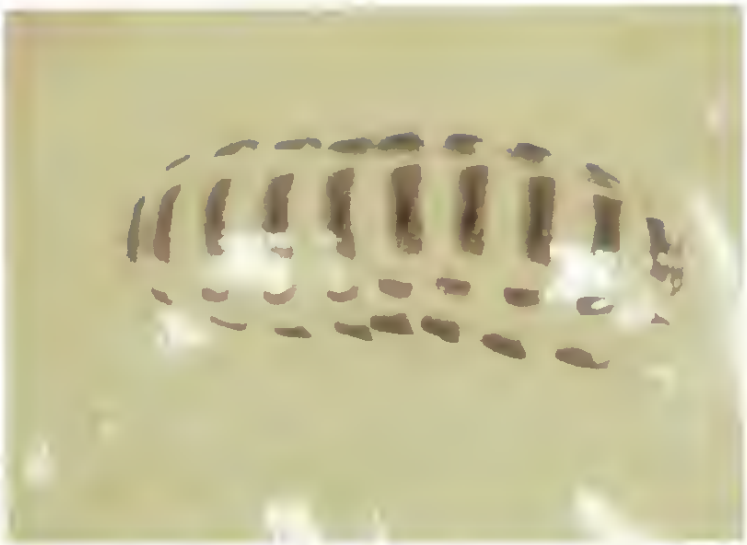

Figure 12: Larva of *O. depleta* in *S. abbreviatus* host two days after inoculation

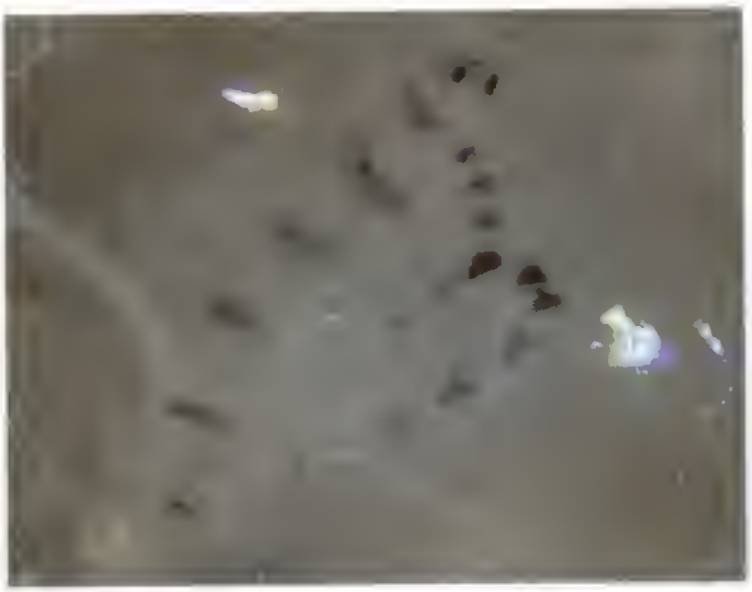

Figure 13: Larva of *O. depleta* in *S. abbreviatus* host three days after inoculation

Figure 14: Larva of *O. depleta* in *S. abbreviatus* host four days after inoculation

Figure 15: *Scapteriscus abbreviatus* host five days after inoculation showing the external evidence of the attachment point of *O. depleta* larva

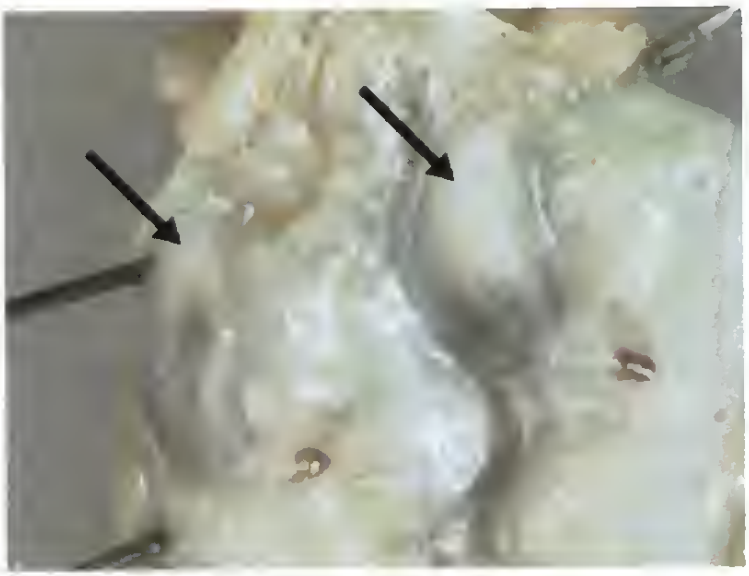

Figure 16: Larvae of *O. depleta* in *S. abbreviatus* host five days after inoculation

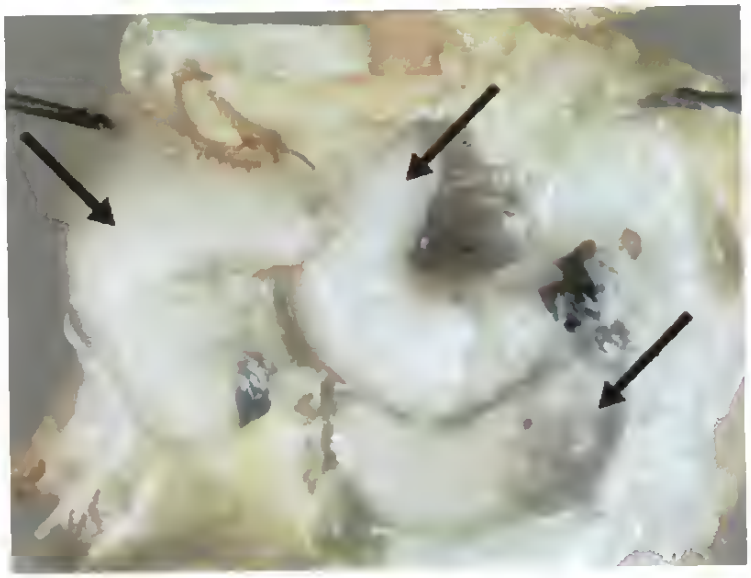

Figure 17: Larvae of *O. depleta* in *S. abbreviatus* host six days after inoculation

Figure 18: Two *O. depleta* larvae in *S. abbreviatus* host six days after inoculation showing detail of the larval attachment to the host

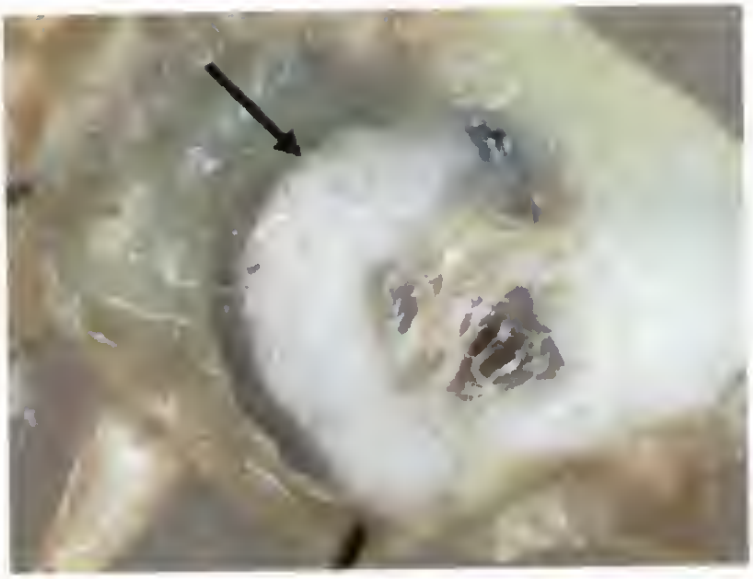

Figure 19: Larva of *O. depleta* in *S. abbreviatus* host seven days after inoculation

Acheta domesticus-

Day 1 (24 hours after inoculation)- Only one larva was found feeding on the fatty tissue just below the area where placed. This larva does not appear to have grown much although it has become slightly lighter in color, most likely due to feeding on fat bodies (Figure 20).

Day 2 – Larvae are easier to see, though still small. They are apparently feeding on fat bodies in the thorax (Figure 21).

Day 3 – Two larvae were feeding on fat tissues near the gonads of the host. One larva was in the anterior region of abdomen (Figure 22).

Day 4 – All larvae found were in the abdomen of the host. All but one were feeding on fat and reproductive tissues. The remaining larva was in the anterior region of abdomen feeding on fat tissue (Figure 23).

Day 5 – Larvae are all now feeding in the gonadal region. Larvae are all now posteriorly attached to the exoskeleton of host. The fat bodies of the host do not appear to be depleted nearly as much as in *S. abbreviatus* hosts (Figure 24).

Day 6 – Larvae continue to feed in the gonadal region. The size of the larvae appears to be increasing, but at a reduced rate as compared to larvae in *S. abbreviatus* (Figure 25).

Day 7 – Larvae are still feeding in the gonadal region. The fat and reproductive tissues of the host appear to be somewhat reduced. Some larvae appear to be showing signs of slowing down or stopping feeding. The alimentary canal of the host is undamaged (Figure 26).

Figure 20: Larva of *O. depleta* in *A. domesticus* host one day after inoculation

Figure 21: Larva of *O. depleta* in *A. domesticus* host two days after inoculation

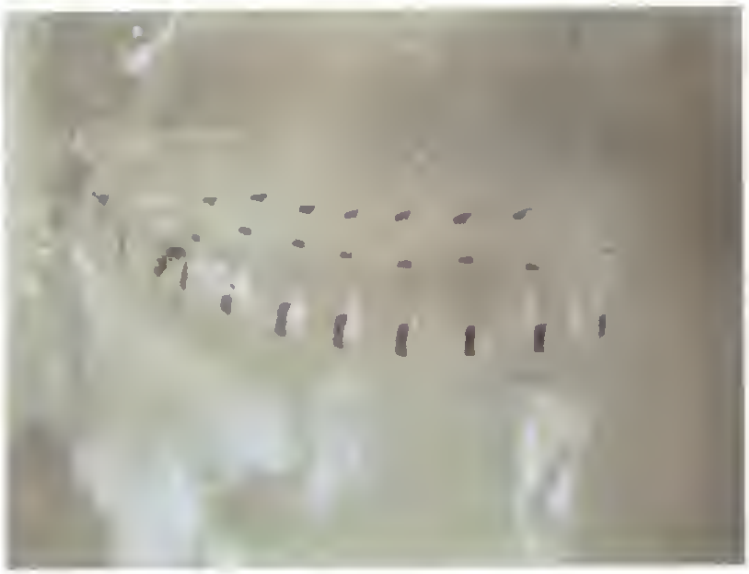

Figure 22: Larva of *O. depleta* in *A. domesticus* host three days after inoculation

Figure 23: Larva of *O. depleta* in *A. domesticus* host four days after inoculation

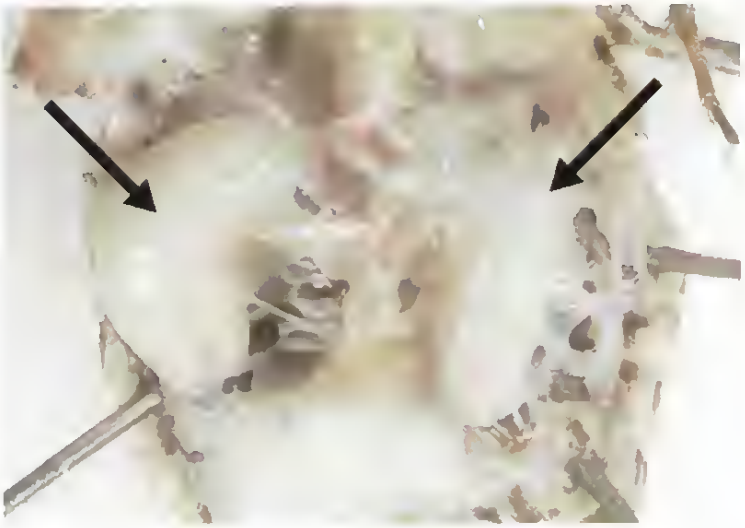

Figure 24: Larvae of *O. depleta* in *A. domesticus* host five days after inoculation

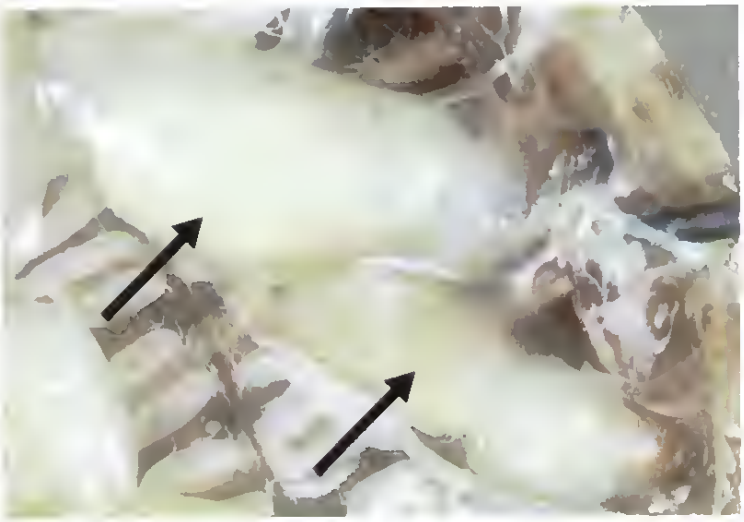

Figure 25: Larvae of *O. depleta* in *A. domesticus* host six days after inoculation

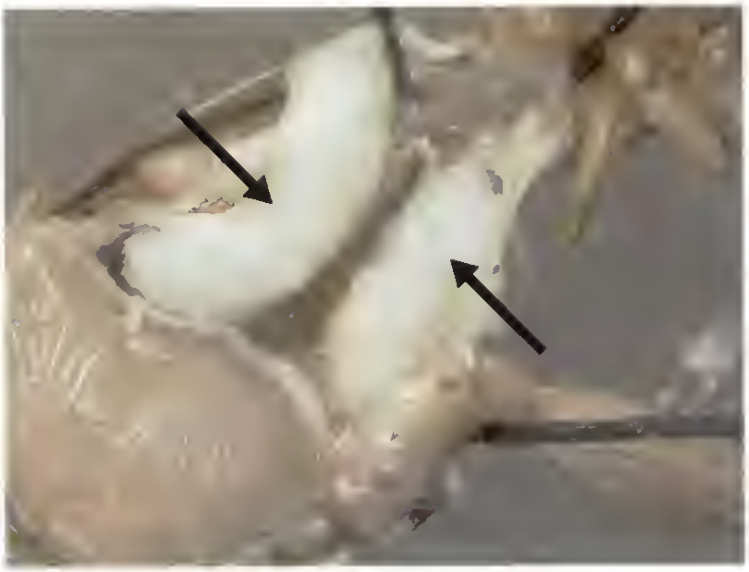

Figure 26: Larvae of *O. depleta* in *A. domesticus* host seven days after inoculation

One larva was removed from each host species on day seven and photographed next to each other to compare the difference between the *S. abbreviatus*-reared larva and the *A. domesticus*-reared larva (Figure 27). The larval length for each day was measured using an ocular micrometer and the mean plotted for each host species (Figure 28).

Figure 27: Seven day old larvae of *O. depleta* from *S. abbreviatus* (top) and *A. domesticus* (bottom)

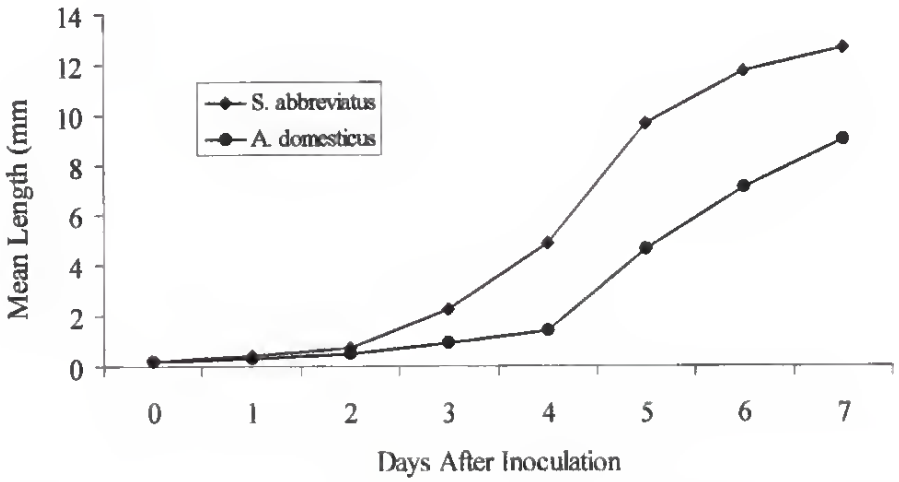

Figure 28: The growth of *O. depleta* larvae in alternate hosts: *S. abbreviatus* and *A. domesticus*

Discussion

Although *A. domesticus* may be easier and less expensive to rear than *Scapteriscus* spp., these results do not support its use as a factitious host for *O. depleta*. For many generations, there is a great abundance of planidia. Therefore, the survival of the planidia is not a weighty factor in determining rearing procedures. Additionally, by

using large numbers of *A. domesticus* as hosts, the number of gravid flies available may be able to be augmented and therefore produce even more planidia. In such a case, a drop from 57% to 29% may not be too damaging to a colony's survival. Considering that this represents approximately a doubling in mortality of planidia, from an economic standpoint this would be acceptable because the cost of rearing a mole cricket host is so much than the cost of rearing a house cricket. The main issue is that of the pupal size. Previous experimentation has shown that for pupae weighing less than 40 mg, there is a reduction in survival (Appendix A). Roughly half of the *A. domesticus*-reared *O. depleta* were in this low-emergence category. This is considering emergence only, no research has been done to determine the effect that this small size may have on survivorship, breeding ability, or fecundity, but it seems likely that any or all of these factors could be affected negatively by significantly reduced weight. Among hymenopterans, reduced female size has been demonstrated to cause a reduction in fecundity in *Trichogramma evanescens* Westwood and *Goniozus nephantidis* (Muesebeck) (Waage and Ng 1984: Hardy et al. 1992). Under laboratory rearing conditions, these problems may be further exacerbated in that the offspring of these under-sized flies would become the parents of the next generation of flies, potentially producing fewer or smaller individuals and reducing the vigor of the colony as a whole.

It is possible that the poor results of *A. domesticus* as a factitious host were due to the techniques used here. The number of planidia used was not altered within this experiment. Perhaps a smaller number per host would have produced larger pupae, although this is not supported by the physiological evidence. There may also be room for improvement of the rearing cages. The 0.6 cm wire mesh used was too large to prevent

the crickets from getting through to the sand. House crickets do not survive well in very moist conditions and this may have affected the larvae. Also, there were problems with keeping the sand moist enough in the well-ventilated cages. Although this would not have an effect on the size or number of pupae produced, in a larger-scale operation this may effect the ability of the pupae produced to survive. Additionally, all of the pupae recovered were found on the surface of the sand and several seemed unusually dry, indicating the inability of the larvae to burrow into the sand effectively. Perhaps in the future a different substrate, such as vermiculite, could be used to better retain moisture.

In considering the larval behavior in the hosts, no single factor stood out as being the cause for the poor performance of *A. domesticus* as a factitious host for *O. depleta.* Rather there appear to be multiple factors. The slowed growth and development of the larvae in *A. domesticus* suggest that there may be a nutritional deficiency associated with *A. domesticus* as a host for *O. depleta.* This deficiency could possibly be a lack of specific proteins or other components necessary or an overall shortage in the volume of palatable or usable tissues. The fact that after seven days there was still a large amount of fat tissue remaining in the host does not support the position that these tissues are simply lacking in volume. It is more likely that they are nutritionally deficient or unpalatable. Another explanation would be that there is a chemical or immunological incompatibility between *A. domesticus* and *O. de*pleta. Further research in these areas is still needed.

Although smaller, the larvae of *O. depleta* in *A. domesticus* do appear to survive as well as those in *S. abbreviatus,* as evidenced by the similarity between the mean number of larvae found in each over the last few days before emerging from the host. There is, however, great reduction in the number of pupae produced by the different

hosts. There must be a cause of mortality between these two events. The most probable cause for this mortality would be a problem with the ability of the larvae to emerge from the host. *Scapteriscus abbreviatus* are subterranean and their bodies are well sclerotized, however, their abdomens are quite soft and the cuticle thin. By contrast, *A. domesticus* have a relatively hard and thick abdominal cuticle. This could very well be a barrier *to O. depleta* larvae being able to emerge successfully. This would especially be true for larvae of reduced size and vigor. Emergence may also be affected by the factors present as the larvae leave the host and are exposed to the laboratory environment. *Ormia depleta* would probably be adapted to emerging from its host into a subterranean environment where the relative humidity would normally be very high. Those larvae emerging from *A. domesticus* would find themselves immediately exposed to much drier conditions. The pupae of *O. depleta* are well protected from desiccation, but before the formation of the pupal sclerotia, the larvae could be extremely vulnerable, perhaps enough to keep it from being able to fully emerge from the host.

CHAPTER 5
IDENTIFICATION OF TWO SOUTH AMERICAN GEOGRAPHICAL ISOLATES OF *ORMIA DEPLETA* BY ANALYSIS OF CUTICULAR HYDROCARBONS

Introduction

With the release of the Osório strain of *O. depleta*, it became desirable to find a way to differentiate the two strains to make it possible to identify the origin of any flies captured. Although no flies have yet been trapped where the Osório strain was released in states north of Florida (personal communication with Dr. J. H. Frank, University of Florida), those flies may perhaps be established in those areas, and in the areas between the new release sites and the established range of the Piracicaba strain of flies. Gas chromatographic (GC) analysis of cuticular hydrocarbons was chosen as a method to differentiate the strains. GC has been used to identify different species, subspecies, and races of insects (Sutton and Carlson 1993) and is a quick and simple testing method.

Materials and Methods

Gravid female *O. depleta* of the Osório strain were obtained from the laboratory colony maintained in the mole cricket rearing laboratory at the University of Florida. Gravid female Piracicaba strain flies were collected from the sound traps maintained at the Gulf Coast Research and Education Center in Bradenton, Florida. Planidia from each strain were inoculated onto *S. abbreviatus* and kept under normal conditions as outlined previously. Colonies of both Osório and Piracicaba strains of *O. depleta* were maintained until maturity (approximately four weeks after pupation). Males and non-gravid females from both colonies were removed and frozen until time for cuticular hydrocarbon

extraction. Both individual and collective samples were taken. Individual samples were collected by placing a single frozen fly in a vial containing 5 mL of hexane and agitating for 30 seconds. The fly was then removed and discarded. The hexane solution was then filtered through a glass pipette containing a 5 cm column of silicic acid. The pipette was then flushed with an additional 5 mL of hexane. The sample was then evaporated down to approximately 1 mL of solution. This process was repeated for eight flies for both males and females from each of the two strains. Collective samples were taken by placing 10 frozen flies of the same sex and strain into vials containing 10 mL of hexane. These samples were also filtered through glass pipettes containing a 5 cm column of silicic acid and flushed with an additional 5 mL of hexane. Samples were evaporated down to approximately 3 mL of solution. Both individual and collective samples were analyzed by gas chromatography using a Hewlett Packard 6890 GC with a DB-5 fused silica capillary column (30m × 0.25μm, J & W Co., Folsum, CA using hydrogen carrier gas @1.2 ml/min and a cold on-column injector system held at 60°C). The temperature ramp for the samples was as follows:

Initial temperature 60° C – hold for 2 minutes

Increase at a rate of 16°C/min to 170°C

Increase at a rate of 8°C/min to 234°C

Increase at a rate of 6.5°C/min to 253°C

Increase at a rate of 5.4°C/min to 320°C

Hold at 320°C for 20 minutes

The purpose for this specific temperature ramp is that it separates straight-chained alkanes into peaks with retention times approximately one minute apart (pers. comm. with Dr. David Carlson, USDA-CMAVE, Gainesville, Florida). The resulting chromatograms were analyzed by stepwise selection in multiple logistic regression to determine discriminatory peaks between strains. Additionally, a jackknife analysis was performed to assess the probability of the resulting model to correctly predict the strain of an unknown sample (SAS Institute 2001).

Results

Ten predominant peaks were present in the cuticular hydrocarbon GCs of all specimens tested. (Figures 29-32) These peaks were designated by the letters A through J and their retention times listed in Table 7. The strongest variation between the two strains was found to be between the peaks of the males, when analyzed by multivariate analysis, with the Piracicaba strain having a higher percentage in both peaks. A stepwise logistic procedure found complete separation of data points when comparing peaks F and I, with the Piracicaba strain having a higher percentage of both peaks. Figure 33 shows a scatter plot of the values for peaks F and I for both strains. The jackknife analysis showed that, based on these data for peaks F and I, the probability of correctly identifying an unknown specimen is dependent upon the strain of that specimen. Osório strain flies were correctly identified with 100% accuracy. Piracicaba strain flies were only identified correctly 60% of the time with the remaining 40% being falsely identified as Osório strain.

Table 7: Retention times of GC peaks for the cuticular hydrocarbons of *O. depleta*

Peak	Retention Time (in minutes)
A	21.00
B	23.00
C	23.50
D	25.00
E	25.18
F	27.00
G	27.15
H	27.20
I	27.35
J	27.45

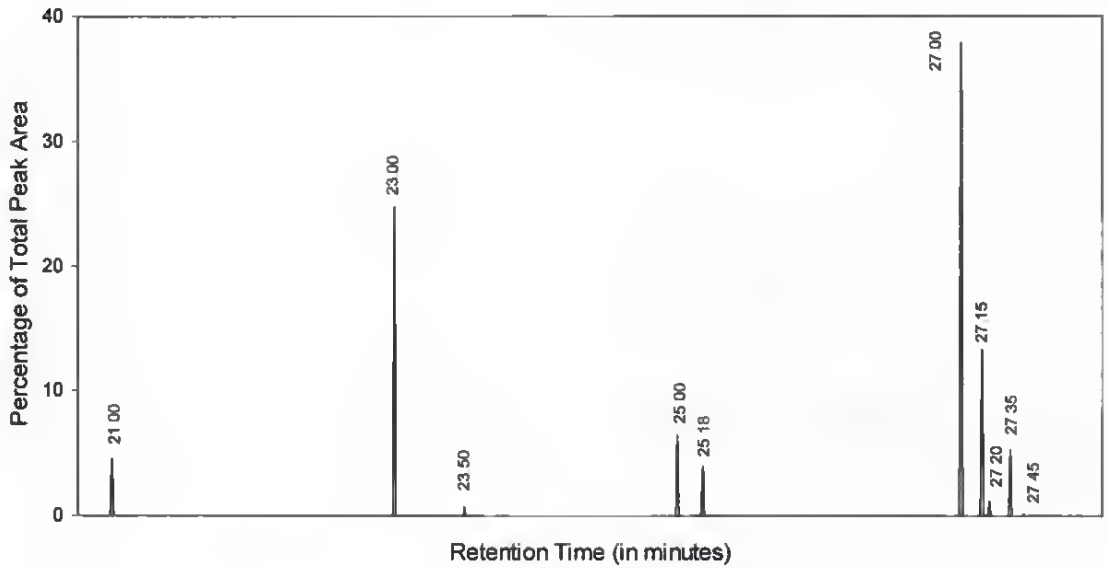

Figure 29: The mean percentage of total peak area for the gas chromatograms of the cuticular hydrocarbons of female Piracicaba strain *Ormia depleta*

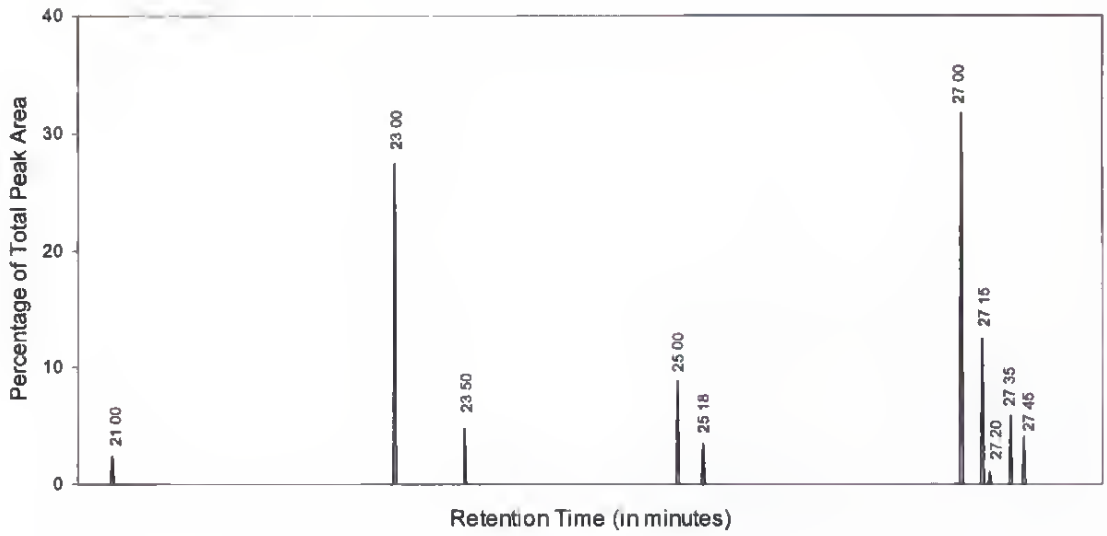

Figure 30: The mean percentage of total peak area for the gas chromatograms of the cuticular hydrocarbons of female Osório strain *Ormia depleta*

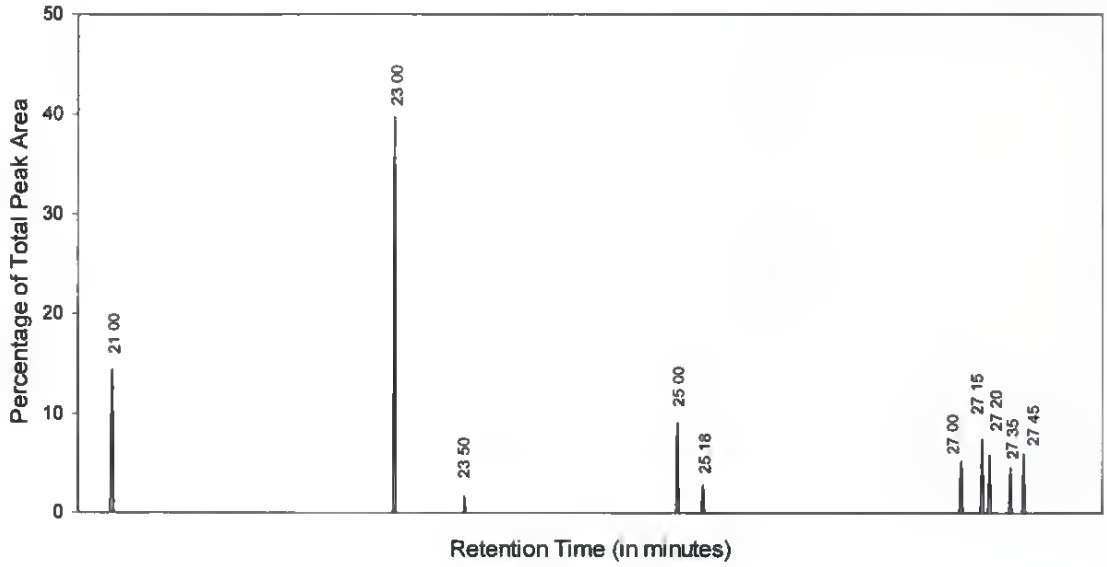

Figure 31: The mean percentage of total peak area for the gas chromatograms of the cuticular hydrocarbons of male Piracicaba strain *Ormia depleta*

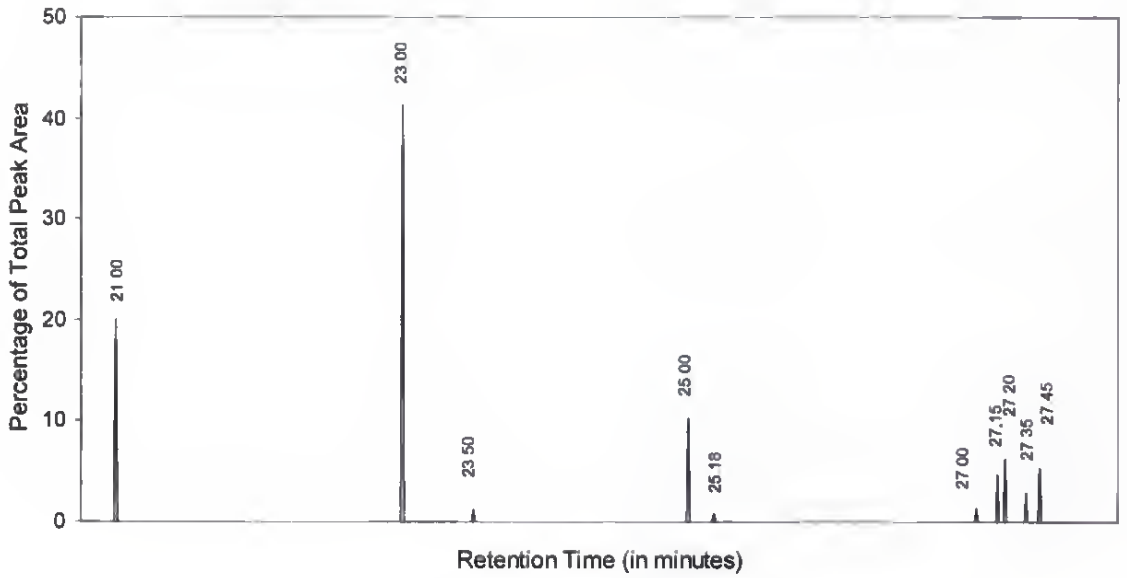

Figure 32: The mean percentage of total peak area for the gas chromatograms of the cuticular hydrocarbons of male Osório strain *Ormia depleta*

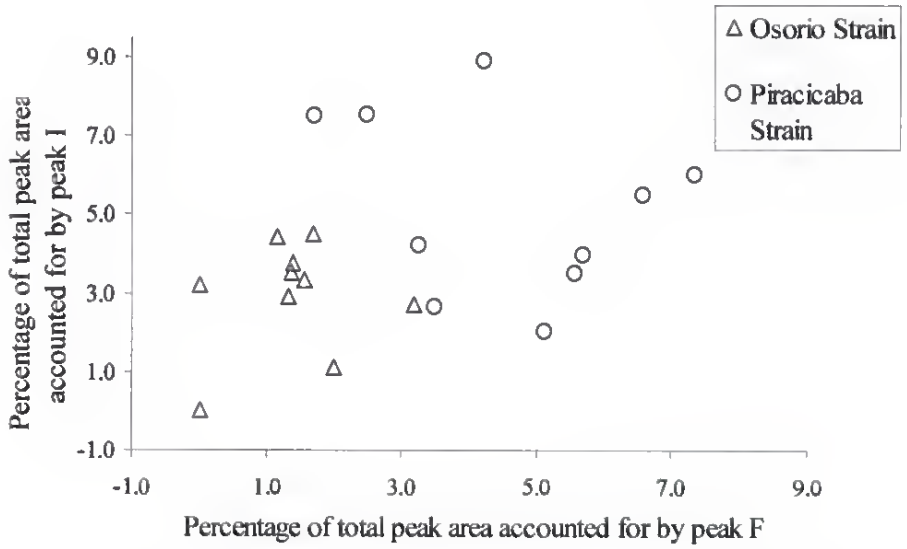

Figure 33: Scatter plot representation of the values for peaks F and I for male *O. depleta* of the Piracicaba and Osório strains

Discussion

The complete separation of data points shows that, based on these data, males of *O. depleta* can be identified to strain by GC cuticular hydrocarbon analysis with the use of only two GC peaks. The difficulty in this lies in that males are rarely, if ever, collected. However, because cuticular hydrocarbon makeup is influenced by a number of factors including the age of the insect and its diet (Liang and Silverman 2000), specimens prepared for GC analysis must be laboratory-reared under the protocol outlined above. Therefore, gravid females collected would have to be used to inoculate mole crickets and their offspring laboratory reared and tested. Because of this, it would be just as easy to test males as females.

The lack of better predictability of identification of unknowns is somewhat disappointing. This failure is most likely due to the rather small sample size with which the jackknife analysis had to work as opposed to the actual differences in the cuticular hydrocarbon makeup of the two strains. A more sophisticated model using more than two peaks may be able to correct this even with the current data size. For this reason, the raw data taken for both strains, as well as a hybrid of the two strains, has been presented in Appendix C.

CHAPTER 6
SURVIVAL OF *ORMIA DEPLETA* WHEN CAGED WITH HONEYDEW-PRODUCING *APHIS NERII*

Introduction

The establishment of *Ormia depleta*, along with *Larra bicolor* and *Steinernema scapterisci*, has been shown to have a strong negative effect on the overall population of *Scapteriscus* mole crickets in Florida (Walker et al. 1992, Parkman et al. 1996). Although *O. depleta* is apparently well established throughout most of central and southern Florida (Frank et al. 1996), there has not yet been established any method to augment local populations of the flies in areas where an increased level of mole cricket control would be desired. Areas such as golf courses, which have a very low threshold for turf damage, could benefit from an increased local population of natural enemies, which would reduce the population of pest mole crickets.

Augmentation of biological control agents has been achieved in many ways. Inundative releases of *O. depleta* are not practical due to the difficulty in rearing them. Banker plants have been used to introduce and disperse parasitoids into field situations (Goolsby and Ciomperlik 1999). Banker plants may also be useful in providing food sources for adult parasitoids and even acting as an attractant. Investigation into determining a suitable banker plant for *O. depleta* led to the discovery that much of their diet consists of homopteran honeydew (Welch 2000). Therefore a plant nectar source would not be likely to make a suitable banker plant. Alternatively, a plant which would reliably maintain a population of honeydew producing homopterans could be established

in areas where a higher population density of parasitoids was needed. If the honeydew produced was attractive to *O. depleta*, and the homopteran population could be maintained, it would very likely achieve the desired augmentation of the local population.

Aphids feed on a number of ornamental plants that may be suitable as banker plants for *O. depleta*. Although almost any plant could be incorporated into various landscapes to attract *O. depleta*, there are considerations to be made when choosing a banker plant. Honeydew production of the aphids associated with each plant is important, as are the host range of those aphids and their pest status. *Pittosporum* spp., *Pyracantha* spp., and *Viburnum* spp. are all infested by *Aphis spiraecola* Patch, but this species of aphid also attacks *Citrus* spp. (Fasulo et al. 2003). *Rosa* spp. are host to the rose aphid, *Macrosiphum rosae* (L.), which may be a suitable banker organism. Roses, however, are not generally the type of plant used in golf course landscape, and encouraging a pest of roses may not be as acceptable as encouraging pests of other ornamentals. *Gardenia* spp. and *Hibiscus* spp. are host to *Aphis gossypii* Glover, but this species is also a pest of *Citrus* spp., making it unsuitable for recommendation. *Camellia* spp. and *Gardenia* spp. are hosts to the green peach aphid, *Myzus persicae* (Sulzer), but as this species has a wide host range and produces a relatively small amount of honeydew, it is not a good candidate (Capinera 2001). The crepe myrtle aphid, *Sarucallis kahawaluokalani* (Kirkaldy), feeds on *Lagerstroemia* spp. and along with the podocarpus aphid, *Neophyllaphis podocarpi* Takahashi, which feeds on *Podocarpus* spp., may prove to be reliable banker organisms for *O. depleta*.

The oleander aphid, *Aphis nerii* Boyer de Fonscolombe, is a minor pest of oleander (*Nerium oleander* L.) and milkweeds (*Asclepias* spp.) (Johnson and Lyon 1991).

These aphids are able to use some of the toxins of their host plants for their own defense (Malcolm 1986), but they are still susceptible to generalist predators and the braconid wasp *Lysiphlebus testaceipes* Cresson (Hall and Ehler 1980). Whether these toxins would be in the honeydew and whether they would act as a deterrent to feeding by *O. depleta* was unknown. Although *A. nerii* colonies can quickly grow to huge numbers on host plants, they do not generally cause a decline in the health of the plant as damage is generally restricted to unsightly sooty mold caused by the secretion of honeydew (Hall and Ehler 1980). Due to this mild pest status, the narrow host range, and the observation that colonies of *A. nerii* are present in Florida throughout much of the year, this species was chosen for evaluation as a potential banker organism to provide honeydew for populations of *O. depleta*. The objective of this experiment was to determine whether *Aphis nerii* honeydew was a suitable diet for *O. depleta*.

Materials and Methods

Pupae of Piracicaba strain *O. depleta* were obtained from the laboratory colony at the University of Florida, which was reared from females collected from the Gulf Coast Research and Education Center in Bradenton, Florida. Six cylindrical rearing cages (30 cm diameter × 60 cm tall) were placed in an outdoor heated greenhouse. Each cage contained a 20 dram vial of moist sand with six *O. depleta* pupae, a small (53 mm diameter) Petri dish of water wicked with cotton, and an approximately 20 cm tall milkweed *(Asclepias curassavica* L.) plant in a 10 cm diameter pot. Milkweed plants in five of the six cages were inoculated with ten adult *A. nerii* each, which were field collected from *A. curassavica* plants, and the colonies were allowed to grow undisturbed for two weeks before exposure to the flies. By this time, each colony was well established

and covered the new growth of each plant. The milkweed in the sixth cage was not inoculated with aphids to serve as the "starvation" control. None of the plants were in bloom at the time of the experiment, so the only carbohydrate source available was from the honeydew secreted by the aphids. At the time of the introduction of the pupae, copious amounts of honeydew were visible on the plants as well as the sides of the rearing cage. The adults emerged over a two-day period. The number of flies remaining alive in the cages was recorded each day, and the experiment ran until all the flies were dead. Each fly acted as a replicate to produce a mean lifespan for each treatment.

Results

The flies that had access to the honeydew of *A. nerii* lived an average of 21.7 days (SD = 7.1, n = 21). The flies without honeydew lived an average of 15.7 days (SD = 4.1, n = 4). A *t*-test showed this difference to be significant ($P < 0.04$). Neither set of flies produced any gravid females.

Discussion

Although the results of this experiment did show significance, the level of significance would have likely been much greater had it not been for several factors. Running this experiment in a heated greenhouse resulted in environmental factors that were not conducive to the health of the flies. The levels of temperature and humidity fluctuated greatly. The mean life span of the honeydew-fed flies was far less than the recorded life span of flies fed other diets in previous experiments. Under laboratory conditions the mean life spans of flies fed hummingbird nectar and melezitose solution were 32.7 days and 39.0 days respectively (Welch 2000). Honeydew-fed flies in this experiment only averaged 21.7 days. Although this may seem to indicate that this particular honeydew is not a suitable diet, the flies with no food only had mean life spans

of 15.7 days in this experiment whereas the mean life span under previous laboratory conditions for starved flies was 22.2 days (Welch 2000). This indicates an overall life span reduction caused by another factor. In this case, the most likely factor was humidity. This experiment took place from December to January and because the greenhouses were heated, the air remained quite dry. Additionally, the host plants for the aphids had been kept very well watered prior to this experiment by placing them in shallow dishes of standing water. This was done to reduce the stress of the aphid load on the plants. When the plants were placed in the cages, they were kept watered, but the standing water dish was removed to keep the flies from drowning. The resulting reduction in available water was apparently the cause of the plants beginning to decline. By approximately day ten of the experiment, all the plants were dead. The experiment was allowed to continue because the amount of honeydew that had already been produced was significant. Honeydew had been heavily deposited on the leaves of the plants, the wall of the cage, and the plant pots. The amount of honeydew that flies were able to consume was enough to produce significant results, but had the plants lived longer, the difference would have likely been more dramatic.

The results do seem to confirm that the honeydew of *A. nerii* is not toxic to *O. depleta* and that it is a suitable food source. Because of the difficulties encountered in this experiment, no real conclusions can be made as to the suitability of *Aphis nerii* honeydew as a food source for the augmentation of *O. depleta* in the field. This experiment was not repeated because the laboratory colony of *O. depleta* died out while it was being conducted and a new colony was not obtained until it was too late to run the experiment

again. The ability of *A. nerii* honeydew to act as an attractant in the field was not investigated here.

APPENDIX A
THE EFFECT OF PUPAL SIZE ON EMERGENCE

To determine if the fitness of the pupae of smaller-than-avaerage *O. depleta* was reduced, a short experiment was conducted. Pupae were divided into 5 groups based on their weight. Each group was then placed in a separate emergence box containing a 2 cm layer of moist sand and covered with a 1 cm layer of moist sand. The boxes were kept at room temperature (approximately 25° C) and when the adult flies had emerged, the number of adults was recorded. The results are shown below in Table 8. There was a

Table 8: The percent emergence of *O. depleta* pupae of varying sizes

Pupal Size	Number of Pupae	Number of Adults	% Emergence
<40 mg	28	20	71.4
40-50mg	31	29	93.6
50-60mg	50	45	90.0
60-70mg	50	46	92.0
>70mg	60	51	85.0

trend toward decline in survival of pupae less than 40 mg. Whether this is due to some genetic or physiological problem or just a function of having less of a buffer from desiccation is unknown.

APPENDIX B
ATTEMPTED INDUCTION OF DIAPAUSE BY SHORT DAY PHOTOPERIOD IN OSÓRIO STRAIN *ORMIA DEPLETA*

An experiment was conducted to determine if diapause could be induced in *O. depleta* by subjecting the pupae and adults to short day photoperiods. The Osório strain pupae were obtained and placed in two large (30 cm diameter × 60 cm tall) rearing cages. The pupae were shallowly buried in moist sand as per the normal rearing technique. One cage was designated as the short-day cage and one as the long-day cage. Both cages were placed near a window that received indirect sunlight. After sundown each day, the short-day cage was covered with black plastic, consisting of two black plastic bags, one inside the other, to block sunlight. In the morning, the long-day cage received sunlight but the short-day cage remained covered until four hours after sunrise. After four hours, the short-day cage had its black plastic removed and it received light until sundown. Both cages were exposed to sundown, as twilight was considered an important mating stimulant by Wineriter and Walker (1990). The pupae and resulting adults were kept under these conditions. After gravid females had been produced, some from each treatment were sacrificed and their planidia used to inoculate *S. abbreviatus*. These mole crickets were then placed in their individual vials of moist sand and put back into either the short-day cage or the long-day cage in accordance with the planidia with which they had been inoculated. After 10 days, the vials which had contained the mole cricket hosts were removed and the pupae harvested. These pupae were then shallowly buried in moist sand as per the normal rearing technique. The resulting pupal duration was recorded for

each individual pupae. The results showed the mean pupal duration for the short-day pupae to be 14.6 days (0.74 SD) and 13.8 days (0.73 SD) for the long-day pupae. A *t*-test showed no difference between the two treatments.

APPENDIX C
GAS CHROMATOGRAPHIC ANALYSIS OF THE CUTICULAR HYDROCARBONS OF ORMIA DEPLETA TO IDENTIFY STRAINS

Raw data showing the percentage each peak contributed to the total peak area.

Strain*	Sample **	Sex	Peak Retention Time									
			21.00	23.00	23.50	25.00	25.18	27.00	27.15	27.2	27.35	27.45
P	C	F	4.27	22.81	0.60	5.98	4.24	1.31	37.56	13.66	2.07	7.51
P	C	F	5.43	29.07	0.84	7.98	3.75	1.21	34.71	11.38	1.08	4.56
P	S	F	4.67	26.87	0.00	7.42	3.22	35.40	12.22	1.48	5.97	0.00
P	S	F	5.29	18.50	0.00	5.00	5.93	43.66	16.47	0.00	3.91	0.00
P	S	F	4.28	26.76	0.00	6.92	3.64	37.21	11.72	1.34	5.63	0.00
P	S	F	4.15	28.74	0.71	7.41	3.41	35.37	11.94	1.25	5.05	0.00
P	S	F	4.02	24.24	0.69	6.64	4.18	37.51	12.66	1.52	5.95	0.00
P	S	F	3.68	24.81	0.81	5.73	3.80	37.39	15.41	1.23	5.16	0.00
P	S	F	3.95	19.49	2.54	5.15	4.06	42.23	15.36	1.26	5.32	0.63
P	S	F	6.60	28.55	0.98	7.71	3.15	33.94	10.30	1.23	4.73	0.00
P	C	M	16.75	36.19	1.57	7.56	0.43	2.48	6.34	11.75	7.53	9.41
P	C	M	17.90	37.24	1.48	7.30	0.46	1.70	4.58	12.57	7.52	9.25
P	S	M	15.91	40.60	1.54	9.22	3.03	5.57	6.46	3.98	3.52	4.76
P	S	M	12.52	42.04	1.29	10.07	3.04	5.69	9.01	4.97	3.99	5.74
P	S	M	13.57	35.53	1.23	8.76	2.08	7.34	7.53	5.60	6.03	9.00
P	S	M	13.32	34.47	1.01	7.55	3.02	6.56	9.56	5.93	5.51	8.39
P	S	M	16.35	47.10	2.42	10.60	2.12	3.48	5.15	3.77	2.65	4.66
P	S	M	11.92	50.00	1.49	10.99	2.90	5.11	5.13	5.09	2.04	3.56
P	S	M	15.80	41.31	2.34	10.23	3.00	3.26	8.71	5.36	4.25	3.96
P	S	M	15.11	26.39	1.61	4.96	3.89	4.22	8.13	11.76	8.92	8.13

*Piracicaba, Osório, or hybrid (F1 generation from Piracicaba females and Osório males)

**Single or Composite

Strain*	Sample **	Sex	Peak Retention Time									
			21.00	23.00	23.50	25.00	25.18	27.00	27.15	27.2	27.35	27.45
O	C	F	3.22	25.91	0.79	8.00	2.09	1.31	36.61	11.18	2.36	8.54
O	C	F	3.18	27.25	1.12	11.19	1.88	0.98	32.79	11.80	1.89	7.92
O	S	F	0.00	23.89	3.58	12.40	12.40	24.58	9.77	1.16	7.05	2.33
O	S	F	3.66	52.78	6.90	7.08	2.54	31.42	12.74	1.42	8.46	0.00
O	S	F	1.75	22.90	10.36	10.83	1.44	26.88	10.28	2.01	11.15	2.40
O	S	F	5.18	16.86	0.72	3.82	2.59	35.57	14.03	2.76	10.69	1.59
O	S	F	1.36	26.02	3.84	9.67	1.43	33.78	13.02	0.00	1.53	7.25
O	S	F	1.35	25.14	5.02	12.34	1.55	33.42	13.75	0.00	0.00	4.69
O	S	F	3.66	23.77	2.62	5.96	2.72	36.00	13.20	0.00	2.12	9.94
O	C	M	20.88	44.61	1.49	10.83	0.79	1.40	4.24	5.39	3.76	6.62
O	C	M	23.24	44.55	1.49	11.16	1.14	1.32	4.04	5.67	2.90	4.47
O	S	M	25.63	42.57	2.65	10.55	0.95	3.19	2.71	3.32	2.69	5.73
O	S	M	16.46	33.75	0.00	7.11	2.47	1.70	9.46	11.17	4.49	6.14
O	S	M	19.96	38.46	0.00	7.87	1.38	1.38	4.97	8.66	3.54	6.21
O	S	M	28.07	42.17	1.89	9.52	0.00	1.99	1.63	2.31	1.09	2.65
O	S	M	18.27	40.80	0.00	8.96	0.00	1.57	6.61	10.43	3.34	5.30
O	S	M	17.16	46.53	3.11	15.75	0.00	0.00	0.00	1.44	0.00	3.16
O	S	M	18.52	41.76	1.79	11.69	1.07	1.16	3.89	4.23	4.41	8.03
O	S	M	16.27	44.64	0.00	10.09	0.00	0.00	8.02	8.14	3.21	4.37
H	S	F	3.80	12.04	0.60	3.61	5.76	47.91	14.85	1.10	3.14	0.00
H	S	F	3.25	18.88	0.51	5.61	4.66	42.55	14.78	1.17	4.41	0.00
H	S	F	2.82	24.89	0.00	6.86	4.13	43.40	6.98	1.72	6.02	0.00
H	S	F	3.37	22.13	0.62	6.50	2.98	42.76	6.19	2.44	8.14	0.00
H	S	F	4.00	14.89	0.00	4.25	6.31	43.69	18.73	1.11	4.06	0.00
H	S	F	2.01	20.28	0.53	6.54	3.49	40.77	7.64	2.51	11.10	1.14
H	S	F	3.05	22.34	0.80	6.92	2.98	42.24	5.94	2.43	8.33	0.00
H	S	F	3.82	21.91	0.00	5.51	3.52	42.53	10.24	2.04	7.20	0.00

*Piracicaba, Osório, or hybrid (F1 generation from Piracicaba females and Osório males)

**Single or Composite

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BIOGRAPHICAL SKETCH

Craig Hinton Welch was born on February 15, 1971, in Knoxville, Tennessee, to Sara H. and Robert N. Welch. He grew up in Dover, Delaware, where he attended school and spent summers at a YMCA camp in North Carolina where his parents worked as administrators and his interest in biology began. In 1986, his family moved to Salisbury, North Carolina, where he graduated from high school in 1989. He then went on to earn his Bachelor of Science degree in biology from the University of North Carolina at Charlotte. He married his wife Celeste on May 13, 1995. In 2000, he earned his master's degree from the University of Florida in entomology. He and his wife have four daughters: Brooke, Courtney, Natalie, and Valerie.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. Howard Frank, Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Eileen Buss
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Bijan Dehgan
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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